ERRATA

Page 16, line 4 from bottom, for ''thorough'' read through.
Page 36, line 21, for presures read pressures.
Page 36, line 23, for presure read pressure.
Page 41, line 11, for coresponds read corresponds.
Page 68, reference 38, for exacten read exakten.
Page 69, line 11, for mongraph read monograph.
Page 124, experiment no. 16, for last figure + 11 read + 101.
Page 124, experiment no. 16, for average + 41 read + 53.
Page 124, experiment no. 17, unexposed side, eighth column, for 43 read 48.
Page 153, line 8, for indicates read indicate.
Page 192, line 13, for are read is.
Page 208, line 4, for easly read easily.
Page 208, line 11 from bottom, for activited read activated.
Page 222, line 9, for was read were.
Page 222, line 10 from bottom, for have read has.
Page 313, line 1 of text, for infection read inflection.
Page 413, line 12, for contains read contain.

PLANT PHYSIOLOGY

JANUARY, 1930

THE RELATION OF TEMPERATURE AND THE PARTIAL PRESSURE OF OXYGEN TO RESPIRATION AND GROWTH IN GERMINATING WHEAT*

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(WITH SEVEN FIGURES)

CONTENTS

Introduction

GENERAL STATEMENT.—The present introductory discussion and the experimental study reported in this paper deal with the influence of temperature and of the concentration or partial pressure of oxygen in the surroundings, on the rate of evolution of CO_2 by higher plants in darkness and with adequate water supply.   There is a considerable literature on the relation of higher plants to oxygen supply, and the literature on the influence of temperature and oxygen supply upon respiration in lower plant forms (such as bacteria, yeasts and fungi) likewise is extensive, but with the latter aspect of the subject we need not be concerned here.

Respiration rates may be ascertained in terms of the absorption of oxygen and of the disappearance of the respired plastic materials, such as carbohydrates, etc., but the rate of CO_2 production furnishes the most convenient and practical index of respiratory activity, and it is the index that has generally been employed in experimentation in this field; therefore measurements on CO_2 production will here be generally considered as indications of the respiration rate.   The respiration rate is so dependent on temperature that no very extensive study of the process could be made without the inclusion of temperature among the measured variables and it is necessarily included here.   The influence of light on respiratory activity appears to be slight in all cases where such influence seems to occur, excepting those instances where light operates through the photosynthesis of carbohydrate, in which instances it cannot be studied directly; indeed the experimental problem of the physiological control of respiration in green plants in light is a very special and difficult one.   The water content of the respiring tissues is known to influence respiration very markedly, but only in its lower ranges; it may consequently be left out of account when the respiring material is well supplied with water.   In a similar manner the influence of the partial pressure of CO_2 in the surroundings may be neglected when the gaseous products of respiration are allowed to escape freely so as not to accumulate within the cells to any significant extent.   There are of course innumerable unessential substances that influence respiration, growth, etc., if present in suitable concentrations in the surroundings, their effects being sometimes to accelerate respiration and sometimes to retard it, according to their natures and their concentrations.   Any of these substances may be made the subject of special study after the fundamental temperature-oxygen relations of respiration have been worked out for a given plant material. When such substances are absent or in sufficiently low concentrations it is not necessary to consider them.

After the study here reported had been completed another series of experiments was planned and carried out under similar conditions but in-

cluding a constant partial pressure of ethylene, an unessential substance that has recently assumed considerable importance in respect to the artificial treatment of fruits, vegetables, etc., for the market. The results of the ethylene experiments are reserved for another paper, although some aspects of the problem of ethylene influence are briefly considered in the present contribution.

The process of respiration is fundamental in all vital activity and increased knowledge concerning it is needed in many parts of the field of plant physiology and ecology, including agronomy, horticulture, forestry, etc. The influence of environmental conditions on respiration is inevitably involved whenever the more general processes of growth and development are being studied, as in discussions of seed germination [HUTCHINS, (18)], the behavior of roots in soils [CANNON, (7)], and the behavior of fruits, tubers, roots, cuttings, etc., in storage.

Because the respiration process as a whole is closely related to vitality in general and to the various kinds of growth and developmental changes that occur in a plant (such as enlargement, ripening, etc.), it is always desirable to give some attention to growth and development when respiration is to be studied. This is especially true when germination processes and ripening processes are considered, in which development and respiration are so interrelated as to be incapable of separation, even in theory and definition. Consequently the present paper includes some discussion of the relation of growth to oxygen supply and temperature.

A brief résumé of our knowledge concerning the influence of oxygen concentration and temperature upon the rate of evolution of CO_2 by higher plants in darkness and without water deficiency is presented below.

RELATION OF OXYGEN PRESSURE TO CO_2 PRODUCTION.—KOSTYTSCHEW, in his recent special monograph on plant respiration (25, p. 18–19) confines his review of the literature bearing directly on the influence of oxygen concentration to a single paragraph. His general conclusion is that, except for plants under extreme conditions, as when they are in nearly pure oxygen or in an atmosphere with less than 1 or 2 per cent. of oxygen, the concentration of that element has little effect on the rate of respiration. PALLADIN (32, p. 215) makes the brief statement that the partial pressure of oxygen in the surrounding atmosphere influences plant respiration without changing the value of the respiratory ratio. This statement is probably based on the results of GODLEWSKI's work (12). KOSTYTSCHEW cites the work of DE SAUSSURE, WILSON, JOHANNSEN, and STICH, which seems to show that changes in external oxygen pressure, even to a considerable degree, effect little change in the rate of CO_2 production. But the results secured by GODLEWSKI point to a different conclusion.

In GODLEWSKI's experiments (12) on radish seedlings a gradually diminishing concentration of oxygen was brought in an enclosed space, by the respiration of the seedlings contained therein, and the rate of CO_2 production steadily decreased as the partial pressure of oxygen was reduced. It might be thought, however, that this reduction in respiratory rate may perhaps have been related to accumulation of products of physiological activity as well as to the lowering of the oxygen pressure.

In WILSON's experiments (46) sunflower seedlings gave off as much CO_2 when in a mixture of 1 volume of ordinary air and 4 volumes of hydrogen as they did when in ordinary air. When, however, the seedlings were changed from air to a mixture of 1 volume of air and 19 of hydrogen, the rate of CO_2 production fell from 18.6 mg. per hour to 12.1 mg. per hour, and when these same seedlings were returned to air the rate rose to 17.8 mg. per hour. The time of exposure was from 1 to 1.5 hours. In STICH's experiments (43) on wheat seedlings and with an exposure time of one hour, a change in the concentration of oxygen in the surroundings from 20.8 per cent. (ordinary air) to 2.0 per cent. caused no change in the rate of CO_2 production. JOHANNSEN (19) found that an increase in the total pressure of ordinary air up to 2 and even to 5 atmospheres produced no effect on the carbon dioxide output of pea seedlings. Respiration of pea seedlings in nearly pure oxygen at ordinary pressures was no more rapid than in air; maize seedlings, however, produced CO_2 a little more rapidly when surrounded by nearly pure oxygen. When sunflower seedlings that had been in air were tested for 45 minutes in a mixture of 1 volume of air and 20 of hydrogen there was a decrease in the rate of evolution of CO_2 and when they were returned to air at the end of the 45-minute period the rate increased slightly, but the original rate in air was not regained in three-quarters of an hour after the return. The relative rates for air, air-hydrogen mixture, and air in succession were 14, 9, and 10, respectively.

It is interesting to note that WURMSER and JACQUOT (47) found a very striking dependence of the respiration rate of a kelp (*Laminaria saccharina*) on the oxygen content of the surrounding sea water. For oxygen contents of 5.2, 6.0, 12.0, 20.0, and 26.0 cc. per liter of water, the relative rates of CO_2 production were 1.0, 1.6, 2.4, 3.1, and 6.3, respectively, being approximately proportional to the oxygen content of the water. HEE and BONNET (15), however, in similar studies on higher plants, found only slight increases in the respiration of water-weed (Elodea sp.) and water milfoil (*Myriophyllum spicatum*) for increases in the oxygen content of the surrounding water from approximately 3 to 20 cc. of oxygen per liter. The time of observation for each oxygen concentration was from 3 to 4 hours, and the same individual plant was used throughout the series. In the field of

animal physiology, AMBERSON, MAYERSON and SCOTT (1) found that, for the lobster (*Homarus americanus*), the annelid worm (*Nereis virens*), the king crab, (*Limulus polyphemus*) and the blue crab (*Callinectes sapidus*), the rate of oxygen consumption was directly proportional to the oxygen tension of the sea water surrounding them, for a wide range of oxygen tensions. For a shrimp (*Palaemonetes vulgaris*) and a squid (*Loligo pealei*) the rates of oxygen consumption were directly proportional to oxygen tension of the sea water when this was below 50 per cent., and 30 per cent. of oxygen saturation, respectively. Above these values, however, the oxygen consumption of these two forms was found to be independent of the concentration of oxygen. NOMURA (31) found that oxygen consumption by a holothurian sea-slug (*Caudina chilensis*) was directly proportional to the oxygen tension of the surrounding water. These authors point out that oxygen consumption should be directly proportional to oxygen tension if there is an oxygen deficit in the tissues and if the rate of oxygen consumption is limited by the rate of diffusion of oxygen into the tissues.

Several investigators have found that in very small organisms in which diffusion of gases occurs very rapidly, oxygen consumption does not decrease appreciably with reduced oxygen concentration until a very low concentration has been reached. Below this critical pressure the oxygen concentration appears to limit the rate of functioning of the respiratory mechanism. SHOUP (41) has reviewed some of these studies and has shown experimentally that oxygen consumption by luminous bacteria is independent of oxygen pressure within the pressure range from 152 mm. of mercury (0.20 atm.) down to 22.8 mm. (0.03 atm.). Below the last mentioned pressure the curve for the rate of oxygen consumption as related to oxygen pressure is similar to curves for adsorption of gases at catalytic surfaces, which leads SHOUP to state that "luminous bacteria are so small that oxygen collecting at the catalytic surface of the oxidation mechanism of the cell becomes the limiting factor determining the rate of oxygen consumption rather than the oxygen diffusing into the cell." The respiratory activity of luminous bacteria ceased in pure nitrogen, and in pure oxygen the consumption of oxygen was irreversibly inhibited.

In the experiments on plant forms where no change in respiration was reported when the environmental oxygen pressure was increased or decreased, the periods of observation usually were short. JOHANNSEN's measurements (19) of CO_2 production by sunflower seedlings successively exposed to air, air-hydrogen mixture and air, furnish evidence that the previous experience of the plants was an important condition in determining their respiration behavior in the short time periods employed. In the light of the studies of AMBERSON and his co-workers and of NOMURA, the

results secured by WURMSER and JACQUOT indicate that in the case of kelp, the rate of diffusion of oxygen into the tissues was a limiting factor in respiration on the supposition that the rate of diffusion would be directly proportional to the partial pressure of dissolved oxygen in the water. In the case of invertebrate animals differences in the responses of the several forms were probably determined by internal conditions of a regulatory nature.

If, under a given set of environmental conditions not dependent on oxygen supply, a plant or a plant tissue fails to show a changed rate of respiration corresponding to an alteration in the oxygen concentration of the surroundings, two possible explanations may be suggested. (1) The rate of oxygen supply within the cells may be proportional to the external partial pressure of oxygen but the rate of supply may not be a limiting condition under the circumstances; the lower rate of supply may be adequate, the higher rate producing neither increase nor decrease in respiratory activity. (2) An alteration of the partial pressure of oxygen in the environment may actually result in a change in respiration rate but there may be a marked time lag between the environmental change and the first appearance of the resulting internal effect, the observation interval being too short to show the response. The second suggestion emphasizes the extreme importance of duration in all physiological experimentation, a consideration that will be reverted to farther on.

INFLUENCE OF TEMPERATURE ON CO_2 PRODUCTION.—The literature concerning the influence of temperature on CO_2 production by plants was reviewed by KANITZ (20) in 1915, whose review included other life-processes of plants and animals, and by KOSTYTSCHEW (25, p. 16) in 1924. KANITZ attempted to analyze the relations of plant respiration and temperature in terms of temperature coefficients and pointed out that in many cases the quotient (Q_{10}) of the rate of CO_2 production for a given temperature divided by the corresponding rate for a temperature 10 degrees lower lies between 2 and 3 for some 10-degree ranges. The coefficient is lower, however, for 10-degree ranges in higher regions of the thermometer scale and higher for 10-degree ranges in lower regions of the scale. As FAWCETT has pointed out (11), the value of the 10-degree temperature coefficient for any process that has a temperature minimum and a temperature maximum must vary all the way from infinity (for 10-degree ranges partly below the minimum) to zero (for 10-degree ranges partly above the maximum), and it is not particularly important that the coefficient value is about 2 or 3 for some intermediate ranges. The interesting thing about temperature coefficients in general is not the actual value of the coefficient for any given 10-degree range but the manner of variation of the coefficient with regard

to the position of the 10-degree range in the temperature interval between minimum and maximum.

KOSTYTSCHEW emphasized the point that there appears to be no optimum temperature for the production of CO_2, above which the range is lower with higher temperature, for the rate gradually increases with rising temperature, to a maximum value at which it remains with still higher temperatures, until the thermal death point of the plant is reached. The same author referred also to the stimulating effect of abrupt temperature changes, as indicated by PALLADIN'S work on this subject (**33**). In PALLADIN'S studies, as cited by KOSTYTSCHEW, etiolated terminal buds of Windsor bean (*Vicia faba*) were kept on 10-per cent. sugar solution at low, medium and high temperatures for 3 days. When the rate of CO_2 production was then determined *at the medium temperature*, the rate was greater for the buds that had been kept at the high and at the low temperatures than for those which had been kept at the medium temperature and this had experienced no temperature change just prior to the measurement.

But KOSTYTSCHEW did not mention BLANC'S objections to PALLADIN'S interpretation. BLANC (**5**) pointed out that plant material kept for a time at one temperature is not to be considered as similar internally to material that has been kept for the same time at another temperature. In his own experiments, on etiolated terminal buds of Windsor bean (*Vicia faba*), young leaves of barley (*Secale cereale*) and embryos of ordinary bean (*Phaseolus vulgaris*), he ascertained the rate of CO_2 production for a given medium temperature, then exposed the material for an hour to a lower or higher temperature, and then again ascertained its rate of CO_2 production at the original medium temperature. The results showed a somewhat lower or somewhat higher rate in the second period at the medium temperature, according to whether the intermediate temperature was lower or higher, respectively, than the medium temperature. These observations were interpreted as failing to show stimulating effects for changes of temperature, although they indicate an after-effect of the intermediate exposure. It seems that the distinction among after-effects, lags, stimulations, etc., are very difficult and involved, and it may be best not to attempt any close analysis of the problem here suggested until much more experimentation has been carried out, with due attention given to all the influential conditions.

INFLUENCE OF CO_2 CONCENTRATION ON RESPIRATION.—SPOEHR and McGEE (**42**) found that changes in the CO_2 content of the air surrounding a leaf had a temporary inverse effect on the rate of CO_2 emission. This effect varied in degree with different species. If the CO_2 concentration were maintained at a constant value after a change, the rate of CO_2 emission

finally became about the same as it was before the change. Kidd (21) found that both anaerobic and aerobic CO_2 production were reduced by CO_2 in the surrounding air. When oxygen was present, but in deficient amounts, so that some degree of anaerobic CO_2 production occurred, CO_2 in the atmosphere exerted no retarding effect. In ordinary aerobic respiration, with ample oxygen supply, however, the same quantitative relation was found between aerobic CO_2 production and the CO_2 concentration as between anaerobic respiration and CO_2 concentration. The inhibiting effect of CO_2 in the surroundings on germination of seeds, sprouting of tubers, and ripening of fruits, as found by Kidd and West (22, 23, 24), seems to be associated with its effects on respiration. The magnitude of the retarding influence was influenced by the concentration of oxygen in the surroundings.

INFLUENCE OF UNUSUAL CHEMICAL SUBSTANCES ON CO_2 PRODUCTION AND ON RIPENING PROCESSES.—Increased knowledge of the conditions that influence respiration promises to be valuable, and indeed quite essential, to an understanding of many forms of chemical stimulation, as, for example, the influence of ethylene on the ripening, blanching, etc., of fruits and vegetables, which has recently attracted much attention. The conflicting nature of the results reported by those who have made careful studies on the influence of ethylene suggests that this practical application can become understood and standardized only in terms of the basic physiological processes and rate changes that are involved, notably the phenomena of respiration. Some of the methods employed in treating plant material with this gas surely produce changes in the partial pressure of oxygen and of CO_2 about the stimulated tissues; for treatment in many instances has consisted in enclosing in a tight container the material to be treated, without any continuous flow of the gas mixture through the confined space. The influence of ethylene, or of any other gas, cannot be satisfactorily studied unless the method of continuous gas flow is employed. With suitable continuous flow of a known gas mixture the partial pressure of the gases used may be maintained almost constant and CO_2, or other gases produced, may be constantly removed, avoiding troublesome accumulation. An experimental study on the influence of ethylene upon respiration and growth of young wheat seedlings was carried out in connection with the investigation reported in this paper, but the results obtained, together with some discussion of the general problem of ethylene influence, are reserved for another publication, as has been noted.

INFLUENCE OF OXYGEN PRESSURE ON GROWTH AND DEVELOPMENT.—Among the early investigators who studied the relation of oxygen to growth and development should be mentioned the Swedish chemist, Scheele (38) (who

discovered oxygen independently in 1771), HUMBOLDT (17), ROLLO (36),
DE SAUSSURE (37) and DÖBEREINER (10).   Their experiments dealt mostly
with germination of seeds and growth of seedlings in nearly pure oxygen
and in ordinary air.   Germination was more rapid in oxygen than in ordi-
nary air, but seedling growth was less vigorous in oxygen.   DÖBEREINER
studied the enlargement of seedlings in air with increased and with reduced
total gas pressure.   Barley plantlets grown in air with half the ordinary
total atmospheric pressure were more spreading, softer, and somewhat
shorter than others grown at the same time in air with a total pressure of
2 atmospheres.   There was more guttation in the plantlets grown with low
pressure and this was considered to indicate higher turgidity.   The dif-
ferences in growth were attributed to turgor differences.

This subject was studied more thoroughly in the quarter century between
1873 and 1900, by BÖHM, BERT, WIELER, JENTYS, JACCARD and SCHAIBLE.
BÖHM (6) found that the growth of plants at the expense of reserve ma-
terials was slower in nearly pure oxygen at a pressure of 1 atmosphere than
it was in ordinary air, but growth in oxygen was more rapid (and like that
occurring in ordinary air) if the oxygen tension were reduced until it was
equal to that of the oxygen in ordinary air, the reduction being accom-
plished either by reducing the total gas pressure or by diluting the oxygen
with hydrogen.   BERT (3) concluded that variations in either direction
from the ordinary partial pressure of oxygen as it occurs in the air resulted
in decreased growth, and he attributed these differences in growth rate to
differences in oxygen tension.

These studies of BERT, WIELER, JENTYS and SCHAIBLE were summarized
by PFEFFER (35), who came to the generalization that most aerobic plants
fail to grow in air at ordinary atmospheric pressure when the oxygen content
of the air is less than from about 0.1 to about 3 per cent. by volume.   He
interpreted the effects of considerable differences in the total pressure
of the surrounding air as influences on turgor, thinking that suitably
diminished pressure increased turgor and so tended to accelerate enlarge-
ment, while sufficiently increased pressure at first reduced turgor and
tended to reduce enlargement, though later it might evoke apparently
adaptive responses of an opposite character, which PFEFFER designated as
counter-effects.   As to the influence of partial pressure or percentage of
oxygen in the air about the plant, PFEFFER concluded that sufficiently in-
creased oxygen percentage in air, with the ordinary total pressure, generally
retarded growth, an effect apparently due to the oxygen itself, acting as a
chemical influence.   He pointed out that the cardinal points (minimum,
optimum, maximum) of partial oxygen pressure, are dependent upon other
influential conditions, especially upon the internal ones that are related to
phase or stage of development, previous experience of the plant, etc.

In none of the experiments considered above were nearly all of the important influential variables taken into account. With green plants in the presence of effective light and with adequate supply of CO_2 the partial pressure of oxygen may be supposed to be high, at least in the illuminated green parts, this partial pressure being largely independent of the oxygen conditions of the surrounding air. The partial pressure of oxygen must be much lower in green parts after a period in darkness, and generally lower than that of the surrounding air in any parts into which oxygen does not diffuse at an adequate rate, as in the deeper-lying tissues of succulent leaves, stems, fruits, etc. On the other hand, when the aerial parts of green plants are kept in darkness or in sufficiently weak light for long periods the phenomena of etiolation are likely to lead to great complications in any attempted analysis of the relations between environmental conditions and the rate of enlargement.

HEUMANN's more recent studies (16) did not greatly add to our information on the direct action of oxygen with respect to growth, for the reasons just suggested. His conclusion is that the acceleration of growth brought about by reduced partial pressure of oxygen in the air is caused by oxygen-hunger in the plant. He supported this idea with the observation that the morphological response to low oxygen supply was an increased area of organs, such as leaves, through which the oxygen of the surrounding air penetrates to the plant interior. The effects observed are thus attributed to an oxygen influence on general metabolism rather than to a direct influence on growth itself.

HARRINGTON (14) noted that when wheat seedlings were in an atmosphere containing 36 per cent. of oxygen growth was nearly twice as rapid as when they were in ordinary air, while further increases in the partial pressure of oxygen resulted in slower growth. He did not mention that light was excluded but light influence through photosynthesis may be supposed to have been of small consequence in these experiments, since seedlings in the early stages of their growth are not dependent to any considerable degree on the products of their own photosynthetic process, which is not rapid in these early stages.

It is evident that the relations between oxygen supply and growth in higher plants are very difficult to evaluate. Although the problem of growth-oxygen relations may be simplified to some extent by the exclusion of light in the experiments, yet the complications arising from the occurrence of etiolation cannot be avoided if the tests are continued throughout extended periods of time. The special responses of etiolation may be rendered less important if short experiment periods are employed, or the experimentation may be confined to germinating seeds or other tissues and organs

that may be kept for long periods in darkness without special effects arising from the absence of light. In any event, the influence of oxygen supply or of the partial pressure of oxygen in the surroundings must be studied with reference to as many of the vital processes as possible if we hope to approach this general problem successfully. For first approximations the general metabolic processes may perhaps be considered as summed up in terms of the rate of respiratory output of CO_2, and growth may be superficially estimated in terms of enlargement and other easily observed features of morphological development. Whenever the time period of an experiment in this field is sufficiently long observations should surely be made on both growth and respiration if such observations are at all possible.

THE TIME RELATION OF RESPIRATION AND GROWTH PROCESSES.—The importance of the time factor in physiological experimentation and discussion is suggested by some of the considerations in the foregoing paragraphs. Whenever the influence of conditions determining the rate of a process is to be analyzed it is essential that time be taken into account not only with reference to the measurement of the process rates themselves but also in the quantitative measurements of the influential conditions. Some aspects of this principle have been emphasized by LEHENBAUER (26), FAWCETT (11), HAASIS (13) and others and it is coming to be generally appreciated. Its special bearing in the present study lies mainly in the fact that developing organisms usually alter as they develop, respiration and growth rates increasing or decreasing with the progress of development. The only way in which a rising or falling process rate may be studied is by means of several short test intervals each of which is so chosen as to represent a suitable portion of the longer period during which the acceleration or retardation occurs. If test periods are too long the corresponding mean rates may be misleading, which is also true if total experiment periods are too short. When a rate is found to be altering with time, under maintained external experimental conditions, it is desirable to employ conveniently short, generally successive, test periods and to continue the experiment either till the rate becomes relatively constant, till it passes through a critical stage at which the sign of its acceleration changes, or till its acceleration becomes constant. When a new set of external conditions is brought to bear the behavior of the organism is usually related for a while to both the old set of conditions and the new set and the only way by which such lagging influences may be studied is through the employment of sufficiently short observation intervals in the earlier portion of the experiment period.

The length of the whole experiment period and the lengths of the partial periods employed for the rate tests are surely very important in such experiments as are required in the field here considered, for the rate of CO_2 evolu-

tion from germinating seeds and young seedlings increases with time, even when all external influences are maintained. These time relations are most important when it is necessary to alter the external conditional complex at the beginning of an experiment, as when seedlings are produced under one set of environmental conditions and are then transferred to another set for an experiment.

The last statement implies a consideration that is sometimes expressed by saying that the behavior of a given organism is partly determined by its past experience and partly by its current environment. Past experience, whatever it may have been, has resulted in the present internal conditions of health, vigor, tone, etc., and present behavior must really be considered as wholly controlled by the combination of current internal and current external influences. But we may be allowed to use the expression past experience to imply present internal characteristics as these have been molded by the environment and the organism operating together in the past.

In experiments that involve a change in the environmental complex, whether from preliminary treatment to the experimental conditions or from one set of conditions to another in the course of the experiment, the resulting changes in physiological processes are usually not abrupt and occur more or less gradually after the change in environment. It is therefore very difficult, as has been said, to separate the results of current influences from those of past influences. The immediate need for this sort of analysis may be avoided if we can find means for adequately defining or describing our experimental material at the beginning of an experiment, being as sure as may be that all coordinate experiments start with material having the internal characteristics thus described.

The estimation of the relative importance of past experience, current environment, and apparently adaptive responses can be arrived at most readily by analysis of the progressive changes in process rates which occur after the lapse of different lengths of time subsequent to specific changes in the environmental complex. As an illustration, let the plant material be changed from one environment to a second, temperature and the partial pressure of oxygen being considerably lower in the second than in the first. Subsequent observations might show the effects of several kinds of response as indicated by the respiration rate: The rate might decrease because of the lower temperature or because of the lower oxygen pressure or because of both these changes acting together; the rate might increase because of one or both of the changes and a tendency toward increase or decrease might be masked by a more pronounced tendency in the opposite direction; a tendency toward gradual increase would generally be expected, because the plant material continues to develop; and various apparently adaptive re-

sponses or counter effects might influence the rate as time went on. It is evident, therefore, that the acceleration of the rate of any process would only rarely be constant, and that changes in acceleration occurring under different sets of conditions, and in different test intervals are valuable clues to the separate responses that really occur.

Experimental methods

GENERAL PLAN OF THE EXPERIMENTS.—The experiments described in this paper were carried out in the Laboratory of Plant Physiology of the Johns Hopkins University, between October, 1928, and March, 1929. The writer greatly appreciates the facilities placed at his disposal by the Johns Hopkins University and also the leave of absence granted to him by the Pennsylvania State College, for the academic year 1928–29, through which these studies were made possible. The writer is glad to express also his personal gratitude to Dr. BURTON E. LIVINGSTON, director of the Laboratory of Plant Physiology of the Johns Hopkins University, for sincere interest, for valuable suggestions in the planning of the experiments, and for kindly criticisms on the preparation of this report.

These experiments were designed to take into consideration the difficulties brought out in the preceding introductory discussion. The process rates studied (CO_2 production and growth) were considered as the resultants of the combined action of the internal and environmental complexes of influential conditions. The influential conditions were considered as of two categories: (a) the background or parameter conditions, which were not consciously varied from experiment to experiment, and (b) the experimental variables (temperature and partial pressure of oxygen) which were maintained constant throughout a given experiment or any repetition of it, but differed from one experiment to another. The background conditions included (1) the internal conditions of the seedlings, which were as nearly alike as possible at the beginning for all experiments, being determined by the nature of the seeds and the preliminary treatment (germination procedure) to which they had been subjected; and (2) all external influential conditions excepting the two experimental variables. The background environmental conditions were planned to be alike for all experiments, being maintained nearly constant throughout the period of each experiment. These comprised water supply, salt supply, and light, the last having a value of zero because the cultures were kept always in darkness except for occasional momentary exposures to weak diffuse light for observations. Other influences which were kept constant or nearly so were the length of the experiment period and the lengths of the successive observation or test intervals within this period. For the different consecutive test intervals, how-

ever, the time was really an experimental variable; for example, the seedlings were of course younger in the first test interval than in the second, and the respiration rates in these intervals therefore corresponded to seedlings of different ages and different stages of development.

The main features of the general plan may be summarized as follows. Seeds of a specified stock were germinated by a specified technique and the resulting seedlings were allowed to develop in cultures, for a definite experiment period (46 hours) following a short period for transfer (0.5 hour) and adjustment (1.5 hour). Each experiment consisted of a single culture of 100 seedlings submerged in 250 cc. of standard nutrient solution, exposed to a certain maintained temperature, with a certain partial pressure of oxygen in the oxygen-nitrogen mixture continuously passing through the solution in the culture flask. Five experiments were carried out at the same time, constituting an experiment series. All of the five cultures in any series had the same oxygen-nitrogen mixture, but each differed from the other four with respect to its maintained temperature. Thus the oxygen-nitrogen mixture was the same for all experiments of the same series or repetitions thereof but differed for different series, while any given maintained temperature was employed for only one of the five experiments in any series. Each experiment thus differed from the others with respect to its combination of maintained temperature and oxygen pressure. Beginning at the end of the adjustment period (1.5 hour) the rate of CO_2 production was ascertained for five consecutive test intervals of 4, 6, 12, 12, and 12 hours, respectively, and observations on seedling development were made at the ends of the intervals, especially at the end of the fifth interval, which terminated the experiment in each case. The results show hourly respiration rates (as indicated by CO_2 production) for the several consecutive test intervals of each experiment and also notes on the corresponding growth rates for each experiment and each set of results corresponds to the specified oxygen-temperature complex that prevailed in the experiment from which they were derived, acting in connection with the internal and external background conditions previously mentioned.

THE WHEAT SEEDLINGS.—The wheat seed used in these experiments was of the Nittany variety, a pure-line winter wheat selected by Dr. C. F. Noll, of the Pennsylvania Agricultural Experiment Station. The stock used was of the 1928 crop and was secured from the originator. It was stored in an aerated container at a temperature of about 20°–25°. The experimental lots, selected to include only sound, well-filled grains, showed a germination percentage of about 98 per cent., under the germination conditions provided. Twice as many seeds were germinated for each series of experiments as were required for the five cultures in the series, in order to allow for selection of

seedlings within relatively narrow limits. Thus 1,000 seeds were used for each experiment series, and five cultures of 100 seedlings each were regularly set up at the same time.

The method of germination, which was adopted after some preliminary tests, consisted in placing the thousand selected seeds in 500 cc. of distilled water in a 600-cc. Pyrex Erlenmeyer flask, and aspirating ordinary air through the water rapidly enough to cause a little movement of the seeds throughout the germination period, during which the flask was kept in the 20-degree compartment of the constant temperature apparatus. Temperature, moisture supply, and aeration were thus very nearly constant throughout the germination period and were practically the same for successive lots of seeds. Using the same quantity of water and the same temperature for each lot of 1,000 seeds gave assurance that changes brought about in the water by the soaking and germinating seeds (such as the outward diffusion of materials, which conceivably might influence germination) were of the same kind and occurred at about the same rates in all cases. No attempt was made to control the chemical content of the air aspirated through the water about the seeds; all the work was done in a well ventilated greenhouse in which plants were growing and no poisonous or stimulating gases could have been present in the air in significant amounts.

The length of the germination period was 42 hours, generally with a deviation of not more than a few minutes. In no case was the deviation more than 40 minutes. The temperature chosen for germination, actually about 19.5°, which is near the optimum for germination of wheat, lies at the middle of the range of the maintained temperatures used in the experiments. The mean temperature of the 20-degree chamber was a little below 20°, for there was a slight cooling at night. The successive lots of seedlings were as nearly alike as could be ascertained by ordinary observation, but of course there was considerable observable variability within each lot of 1,000 seedlings; in spite of the preliminary selection the seeds of a lot did not all reach exactly the same stage of germination in the 42-hour period. Nevertheless, the high degree of uniformity of the seeds and the uniformity of the germination treatment made it possible in every case to select the requisite five lots of 100 seedlings each, so that the populations of all cultures throughout the study, whether of the same or of different series, were apparently alike. The limits of development between which seedlings were selected were such that the coleoptile was always exposed, and the rootlets had not yet broken thorugh the coleorhiza.

It was of course conceivable that the lots of seed used for the later series of experiments might have differed constitutionally in some respects from the lots used for the earlier series, for the seed of the later lots was about

4 months older than that of the earlier ones and significant internal changes might possibly have occurred in the storage period. Some of the earlier experiments were repeated near the close of the work and the results of these showed clearly that the stock of seed had not altered significantly, as will be shown farther on when the experimental data are presented.

The standard conditions for germination constituted the environmental complex for the first 42 hours of soaking and germination, from which the subsequent experimental complex differed with respect to temperature, oxygen concentration and salt environment. Only in those particular experiments in which the maintained temperature was 20° and the partial pressure of oxygen was 20 per cent., was the oxygen-temperature complex the same for the experiment period as for the germination. In all other experiments, the seedlings experienced an abrupt lowering or raising either of oxygen pressure or temperature or both, at the beginning of the experiment. The CO^2 pressure in the environment was nearly constant throughout both the germination and experiment periods, CO_2 being removed by the constantly flowing gas stream in both cases, about as rapidly as it was formed. Since the seeds were germinated in distilled water and were in a standard weak nutrient solution for the experiments, all seedlings experienced a change in salt concentration and osmotic relations at the time the experiments were set up. This change, however, was the same for all experiments. The subsequent physiological activities of the seedlings through absorption and excretion, surely altered the solution during the progress of an experiment and the kind and degree of alteration may have been different for different experiments. Some of the experimental data indicate that these sources of discrepancy had no significant effect on the rate of CO_2 production, from the standpoint of this study. Nevertheless it would have been theoretically better if the same standard nutrient solution had been used for germination as for the subsequent experimental cultures, and if a constant flow of nutrient solution had been arranged as well as of the gas mixture. (On the theoretical advantages of continuously flowing solutions for solution cultures see TRELEASE (45) and SHIVE (40). No technique has been developed, however, for maintaining a constant flow both of gas and of solution through a culture from which CO_2 is to be removed and measured as it is produced.

THE MAINTAINED TEMPERATURES USED AND THEIR CONTROL.—The maintained temperatures tested were 10°, 15°, 20°, 25°, and 30° C. Preliminary tests showed that the experiment period and its consecutive observation intervals would need to be inconveniently long for temperatures below 10°, because of the slowness of growth and respiration at such low temperatures, especially for the earlier observation intervals. Consequently no temperatures below 10° were included in this study. Cultures at 30° showed but

little growth and there were some appearances of breakdown in seedlings
held at that temperature two days or longer, while disintegration appeared
in about 36 hours with a temperature of 40° and no growth was evident at
that temperature.   Since this study was not to deal with lethal and *post-
mortem* phenomena, the range of temperatures tested did not extend beyond
30°.   Temperature differences of 5 degrees were used partly because of con-
venience and partly because they were about as small as were warranted by
the magnitudes of the temperature influences that were observed.

The battery of temperature chambers at the Hopkins Laboratory of
Plant Physiology [LIVINGSTON and FAWCETT (28), HAASIS (13)] consists of
a linear series of seven chambers with water jackets, separated from one
another by uninsulated sheet-metal partitions, but insulated above, below
and at the sides.   The top of the apparatus is formed by the insulated
wooden covers of the water baths.   These are removable, but for convenience
in manipulation, each is provided with three $\frac{1}{2}$-inch holes, through which
tubes leading to cultures, etc., may pass, or thermometers may be inserted,
and also with a rectangular opening (23 x 28 cm.) closed by means of a
removable insulated lid.   Heat is supplied at one end of the series from a
thermostatically controlled hot tank and removed at the other end by means
of a cold tank thermostatically controlled by a mechanical refrigeration
machine.   Each bath is furnished with a mechanical stirrer, which slowly
rotates in the water jacket around the cylindrical chamber, and all parts of
each chamber are at practically the same temperature, but the temperature
of each chamber differs from that of the adjacent chamber or chambers to a
degree determined by the settings of the two thermostats.   The temperature
of the greenhouse in which the series of chambers stands was subject to fluc-
tuations as great as 15° within a period of twelve hours or less and such
changes in the temperature of the external air influenced the chamber tem-
perature to some extent, in spite of the insulation, especially that of the
chambers near the middle of the series, where the daily fluctuation, though
ordinarily less than 1.0° was occasionally as great as 2.0°.   The temperature
of the middle chamber (20°) was usually from 0.5 to 1.0° too low for about
8 hours each night, and the average temperature of that chamber was really
about 19.5°.   Only five chambers were used in this study, the two end ones
of the series of seven being idle.   The chambers used were operated to give
maintained temperatures close to 10°, 15°, 20°, 25° and 30°, as has been
indicated.

Temperature records were made in the 10-, 20-, and 30-degree chambers,
by means of small Richard thermographs, which were kept there throughout
the experiments.   The temperatures of the other two chambers were esti-
mated by interpolation, with occasional direct observations by means of ordi-
nary thermometers in these chambers.

THE GAS CONTROL.—The desired partial pressures of oxygen were secured by mixing compressed commercial oxygen and commercial nitrogen in a gas cylinder such as commercial gases are supplied in, the capacity of the cylinder being well above 2,000 pounds per square inch. A given mixture was prepared by connecting charged cylinders of nitrogen and oxygen successively to a previously discharged cylinder, by means of a copper tube (about 75 cm. long) provided with a suitable coupling at either end and with an ordinary pressure gauge closing a T-outlet in the middle. Each gas was allowed to flow into the mixing cylinder until the gauge gave the required reading, the proportions being measured in terms of pressure. A mixture of 10 volumes of oxygen and 90 volumes of nitrogen was secured, for example, as follows: Oxygen was first allowed to enter the mixing cylinder till the gauge showed a pressure of 100 lbs., after which the valve of the mixing cylinder was closed and the oxygen supply tank was removed. Then nitrogen was allowed to flow into the mixing cylinder till the gauge showed a pressure of 1,000 lbs., after which the valve was again closed and the connecting tube was removed. The result was a tank of gas mixture of practically the required proportions and with a pressure of 1,000 lbs. per square inch. A tank of gas mixture might be kept closed till needed, when it was connected to the gas line leading to the culture flasks.

The compositions of the commercial gases and of the mixtures containing less than 30 per cent. of oxygen were ascertained through absorption of the oxygen by phosphorus. In the analysis of commercial oxygen a small amount of the gas was introduced into the eudiometer and its volume was measured, after which commercial nitrogen (from which the oxygen had previously been absorbed by means of phosphorus) was added in quantity sufficient to reduce the percentage of oxygen to less than 50 per cent. The mixture thus formed was then analyzed and the necessary computations were made.

The gas mixtures were delivered into the tubing lines leading to the culture flasks at a pressure regulated by means of a Hoke Phoenix Regulator or pressure-reducing valve. The maintained rate of flow through a flask was determined by the pressure of the supply and by an orifice control in each tube. Before reaching the separate lines leading to the cultures, the gas was passed first through a tube (of 18-mm. bore and 30 cm. long) containing soda lime, to remove any CO_2 which might be present, and then through concentrated H_2SO_4, to remove moisture, which might have tended to clog the orifice controls. The gas was then delivered to the separate lines through a 5-branched manifold of copper tubing.

The orifice controls were similar in principle to those described by HUTCHINS (18). Each consisted of a section of glass tube (of 5-mm. bore

and 12 cm. long) with a slight constriction about 3 cm. from one end and a plug of alternate layers of dry cotton and powdered chalk packed in the longer portion, against the constriction. The gas passed through the plug before reaching the constriction and the pressure of the gas thus tended to hold the plugs firmly in position. Alternate layers of cotton and chalk were found to be more easily adjusted than a single mass of kaolin or chalk between two cotton plugs, which was employed by HUTCHINS. The controls were adjusted so that the gas flow was 3 cc. per minute for each pound of pressure registered on the gauge of the pressure-reducing valve. The rate of gas flow (measured by means of a eudiometer) was found to be directly proportional to the pressure with this arrangement and it could therefore be controlled at will by setting the valve for the proper delivery pressure. The orifice controls required one or two slight adjustments in the first few days of use, perhaps because of the drying of the cotton or chalk. After these preliminary adjustments, the standard ratio of flow rate to gauge reading remained constant as long as the controls were in use, as was shown by occasional tests.

Between the orifice control and culture flask the gas was led through a telltale, which consisted of a rubber-stoppered bottle with inlet and outlet tubes arranged so that the gas bubbled through about 50 cc. of very dilute barium hydroxide solution colored with a few drops of phenolphthalein. Each telltale stood alongside the culture flask it served, in the same temperature chamber, and the incoming gas was here brought to the temperature of the culture chamber before entering the flask. The constant pink color of the solution indicated the absence of CO_2 in the gas stream. Also, moisture was added to the gas here, so that the volume of the nutrient solution in the culture flasks was not significantly altered by evaporation or condensation.

The total gas pressure above the solution in the culture flasks was always slightly higher than the prevailing atmospheric pressure, because of the hydrostatic pressure of the solution in the absorption-tower and in the telltales, but this difference was considered as negligible, especially since the pressures were the same for all cultures in a given series of experiments. The partial pressure of CO_2 in the culture flasks was kept practically at zero, by sweeping out the CO_2 as rapidly as it was formed.

The rate of gas flow through the culture flasks was established at 20 cc. a minute, after a number of preliminary experiments on the effect of variation in the rate of gas flow. In these preliminary experiments a gas mixture high in oxygen was used, and the rate of flow was different for successive experiment series, with differences of 5 cc. or less for a range from 5 cc. a minute to 20 cc. The gas mixture was presumed to be nearly pure oxygen,

but was not analyzed. It was subsequently indicated, however, to contain about 85 per cent. of oxygen by comparison of results with it and with mixtures the composition of which was known. Experiments were performed also with a mixture containing approximately 20 per cent. of oxygen, some with a rate of gas flow of 5 cc. a minute and some with a flow of 20 cc. a minute. In the experiments with a high percentage of oxygen, the rate of CO_2 production was greater for increased rates of flow up to 15 cc. a minute, but showed no significant changes for higher rates. Growth at the higher temperatures also increased as the rate of gas flow was raised, up to 15 cc. a minute, but did not change for rates greater than this. The proportional increase in CO_2 production for an increase in rate of gas flow from 5 to 20 cc. a minute was found to be practically the same for seedlings in 20 per cent. of oxygen as it was for seedlings in the high percentage of oxygen. This increase in CO_2 production for greater rates of gas flow was probably due to more thorough mixing of the nutrient solution, which resulted in a more uniform supply of oxygen to all the seedlings and more rapid removal of CO_2 from them. The lowest rate of gas flow supplied oxygen to the culture at a rate far in excess of oxygen consumption by the seedlings, as indicated by their CO_2 output. The higher rate of gas flow resulted in fairly rapid convection throughout the nutrient solution; a droplet of India ink became evenly dispersed throughout the solution in less than a minute when the gas flow was 20 cc. a minute.

The rate of gas flow therefore was established at 20 cc. a minute, since this rate was evidently more than sufficient to maintain CO_2 production and growth at maximum rates, as far as oxygen supply was concerned, for all the temperature-oxygen combinations tested.

THE CULTURE FLASKS.—The culture flasks were of Pyrex glass, of the Erlenmeyer form, with capacity of 300 cc. Two sets of five were used, so that one set could be cleaned and prepared for an experiment while the other five were still in use. Each flask had a tightly fitting 2-hole rubber stopper through which the inlet and outlet tubes were led. Because flexibility was desirable these tubes were of lead, but the metal did not come in contact with the culture solution at any time. The lead portion extended only about 15 mm. below the stopper, and the inlet tube was extended by a glass portion, attached by an ordinary rubber-tubing coupling, nearly to the bottom of the flask. The entering gas mixture bubbled through the culture solution, and passed out through the other tube. The outlet tube led directly to its own absorption apparatus.

To avoid any possibility of leakage the stoppers of the culture flasks (and also the stoppers of the telltale bottles) were secured by means of special clamps. A horizontal triangle of $\frac{1}{8}$-inch brass plate with an opening in its

center (to accommodate the inlet and outlet tubes) rested on the top of the rubber stopper and was connected with a base piece by means of three vertical 3/16-inch brass rods threaded at either end and provided with brass nuts (from discarded electric dry cells). The base piece on which the flask stood was a horizontal hexagon (about 2.5 cm. thick) of 2-ply wood with three holes near its edge, for the vertical rods. When the clamp was in place and the nuts were tightened the two plates were drawn toward each other and the stopper was held firmly in position in the neck of the flask.

TUBING AND CONNECTIONS.—Most of the tubing lines were of glass, with wired couplings of thick-walled rubber tubing, the joined ends being in contact. Where flexibility was required lead tubing was employed, it being joined to the glass sections by wired couplings of rubber. There were no joints between the orifice control and the absorption apparatus excepting those formed by the rubber stoppers of the culture flask and the entrance telltale, which were very tight. All lead tubing used was first tested for pin-holes, etc., by applying a gas pressure of 20 pounds per square inch with the tubing under water. The tubes for liquid were of glass or flexible rubber.

THE ABSORPTION APPARATUS.—The absorption apparatus embodied some of the principles of an apparatus described by CARRICK (8), with modifications to allow titrations to be made within the apparatus. The details of construction are shown by the diagram of figure 1. The essential features are: the absorption tower, A; the standard acid burette, B; the titration bottle, G; the vent, E, guarded with the soda-lime tube D; the siphon outlet, I; the inlet for standard alkali solution (or water for washing), J; the outlet telltale, M; an additional vent, K, and the outlet, L, both guarded with soda-lime tubes; and the gas inlet tube, F, connecting the apparatus with the culture flask, from which it is the outlet tube. A complete absorption apparatus was provided for each of the five culture flasks, being permanently mounted on a shelf at the side of the constant temperature chamber in which the culture flask was located.

The absorption tower (A, fig. 1) consisted of a heavy-walled glass tube (18 mm. in inside diameter and 55 cm. long) containing a column of glass beads (3–5 mm. in diameter) about 35 cm. high, the beads resting on an inverted glass vial (C) about 7 cm. high, with 5 or 6 holes blown in its bottom. Both vial and beads were found to be required for complete absorption of the CO_2 brought from the culture flask in the gas stream. The inverted vial provided a reservoir of absorbing solution, into which the entering gas bubbles were discharged and in which they were held for a short period, until succeeding bubbles caused the trapped gas to move upward through the holes and among the beads.

The outlet telltale (M, fig. 1) was simply a test tube (20 x 150 mm.) provided with a 2-hole rubber stopper and inlet and outlet tubes, so arranged that the gas escaping from the absorption tower bubbled through a measured quantity of standard barium hydroxide solution before being discharged. The outlet of the telltale was of course guarded by means of a soda-lime tube. Absence of any white precipitate, in this telltale, which was in plain sight, made it certain that no CO_2 had found its way through the absorbing tower.

When absorption of CO_2 was in progress, all of the three rubber connections marked X on the diagram (fig. 1) were closed with Hoffman clamps. A definite quantity of standard barium hydroxide solution (0.2 normal) was measured from a charging burette into the absorption tower through the solution inlet (J, fig. 1) and was washed down with a small quantity of CO_2-free water. The pressure of the gas entering through the gas inlet (F, fig. 1) held the alkali solution in the tower and the gas bubbles rose through this solution, being delayed and broken up by the vial (C) and the glass beads. Thence the gas stream was conducted through the outlet telltale (M) and was finally discharged through the discharge outlet (L).

When a determination was to be made, at the end of a test interval, the vent (K) at the top of the absorption tower was opened first. A few drops of phenolphthalein solution was introduced through the solution inlet (J) and a tube leading from a reservoir of distilled water above the apparatus was then connected to the solution inlet. The vent (E) on the titration bottle was then opened and the solution in the tower was drained into the titration bottle (G) through the tube H. Water was then allowed to flow in through the solution inlet (J) until all traces of pink color had been washed down. The excess of alkali was then determined by titration with standard HCl solution (0.1 normal), from the burette B. The connections were sufficiently flexible to allow shaking of the titration bottle (G), during the titration. When the end-point was reached the neutralized solution was allowed to flow out through the discharge siphon (I), by opening the clamp IX. The siphon tube itself was not emptied, since the water in it provided an insurance against leakage past the clamp. Tower and bottle were finally washed with distilled water, and a new charge of standard alkali solution was introduced as at the beginning, all clamps being properly adjusted.

THE STANDARD ACID SOLUTION.—The standard acid solution used for titration and for standardizing the standard alkali solution was approximately 0.1 normal (0.1132 N) HCl. This was standardized originally against dry sodium carbonate, with methyl orange as indicator, and the standardization was repeated from time to time during the progress of the experimental work, to ascertain whether changes occurred in the concentra-

tion of the acid under the conditions of storage. Eighteen liters of the standard acid solution were prepared at the beginning of the work, and this was sufficient for the entire series of experiments. This stock was kept in an 18-liter bottle closed with a 2-hole rubber stopper carrying a glass siphon for withdrawal of solution and an air inlet, the latter protected by a soda-lime tube to exclude CO_2. Two 4-liter reservoir bottles were filled from this stock, and were kept on an overhead shelf suspended from the frame of the greenhouse. Standard acid solution was delivered to the acid burette (B, fig. 1) of each absorption apparatus by means of glass tubing with rubber connections for flexibility. The reservoir bottles were stoppered in the same way as was the large stock bottle.

THE STANDARD ALKALI SOLUTION.—The standard barium hydroxide solution was prepared by dissolving "Baker's Analyzed" barium hydrate crystals in distilled water at the rate of 31.554 gm. per liter of solution with addition of 4.5 gm. "Baker's Analyzed" barium chloride per liter. The latter was added to reduce the solution and hydrolysis of the $BaCO_3$ which was present at the time titrations were made. An 18-liter stock bottle of this alkali solution was prepared at the beginning of the experiments, and this was sufficient for all of the work. The bottle was closed like the stock bottle for the standard acid solution. The small amount of barium carbonate present (there is usually some carbonate in barium hydroxide) was first allowed to settle and then the clear solution was siphoned into reservoir bottles like those used for the standard acid solution and similarly placed. From these reservoirs the solution was delivered to the charging burettes by means of a branching system of glass tubing with short rubber connections for necessary flexibility, as shown by the diagram in figure 2, described below. All air inlets into the distributing system for standard alkali solution were provided with soda-lime tubes, to prevent the entrance of atmospheric CO_2. That no CO_2 entered the system was shown by repeated tests of the solution as delivered by the burettes, titrations being made with standard acid solution and phenolphthalein.

CHARGING THE ABSORPTION APPARATUS.—The charging burettes were arranged to receive and deliver standard alkali solution without its coming into contact with atmospheric CO_2. Their arrangement is shown in figure 2. The supply tube (T) leading from the reservoir bottle (R) is connected to the recurved tube (C) of an automatic burette (B) through the 2-way stopcock (CA). Solution flows into the burette when the cock is in the position shown and when the cock is turned half-way around solution is discharged through the tip A. Air is allowed to escape from or enter the burette through the opening above, which is fitted with a 1-hole rubber stopper bearing a soda-lime tube (G).

When an absorption tower was to be charged with alkali solution a small amount of solution was first allowed to run out of the burette into a beaker, to remove what had been in the tip (A), in contact with the air. The burette was then lowered slightly and the tip was connected immediately with the solution inlet of the absorption tower (J), after which the desired amount of solution was allowed to run into the tower. After closing the 2-way cock and disconnecting and raising the burette a small amount of CO_2-free water was introduced into the solution inlet before this was closed, to carry down all the solution measured from the burette. It was found that the possibility of error was reduced if the excess of standard alkali solution did not greatly exceed the equivalent of 3 or 4 cc. of the standard acid solution.

Repeated checks were made in the following manner, to test the accuracy of the method of titration within the apparatus. Some of the $BaCO_3$ from a previous experimental test was allowed to remain in the titration bottle (G, fig. 1), after the excess alkali had been neutralized and most of the liquid had been siphoned out. A measured quantity of standard alkali solution was then introduced into the absorption tower in the manner described above, and was washed down into the titration bottle with distilled water after a few drops of phenolphthalein had been added. The water for washing was introduced into the top of the tower by connecting a tube leading from a reservoir of water on the shelf above the apparatus, to the solution inlet (J, fig. 1). Ordinarily a total of 150 cc. of water, introduced in several portions, was sufficient to remove all traces of alkali from the tower, as indicated by the disappearance of pink color from the beads in the tower. The alkali solution thus introduced into the titration bottle was titrated immediately with the standard acid solution. The results of these titrations in the apparatus, in the presence of the $BaCO_3$ mentioned above, agreed with other results obtained with a similar quantity of the standard alkali solution, taken directly from the charging burette (B, figure 2) into a flask containing 150 cc. of distilled water, and titrating immediately with standard acid solution from a burette separate from the apparatus, using phenolphthalein as an indicator. This agreement of results indicates that $BaCO_3$ is not a source of error in this sort of titration, [which is in agreement with statements by TREADWELL and HALL (44, p. 485) and BERGMAN (2).] These repeated tests, both inside and outside the apparatus, served also as checks on the constancy of the standard alkali solution.

TITRATION.—As is evident from what has been said, titration was accomplished in the presence of the $BaCO_3$ that had been formed by the reaction of the absorbed CO_2 and the $Ba(OH)_2$ of the alkali solution in the absorp-

tion tower.   At the end of a test interval, the solution in the tower was
washed down into the titration bottle (G, figure 1), in the manner previously
described.   Very nearly the same amount of distilled water was used in
each case.   The water for washing was guarded against contact with atmos-
pheric CO_2 by a soda-lime tube in the air inlet of the water-reservoir bottle.
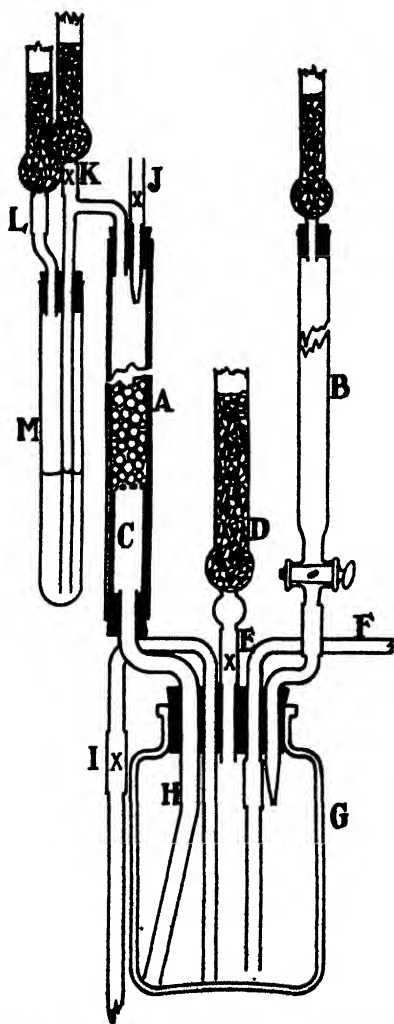
FIG. 1.   Diagram showing arrangement of parts of CO_2-absorption apparatus.   For de-
scription see text.

Standard acid solution was added drop by drop, with constant shaking of
the titration bottle, to avoid any local excess of acid, which might react with
the $BaCO_3$.   During the process of washing down and titrating, which

required about five minutes, the gas stream continued bubbling through the solution in the titration bottle.   Consequently the only loss of CO_2 which occurred took place while the neutralized solution was being discharged and the apparatus was being washed, preparatory to the addition of a new charge of alkali solution.   This slight loss was obviously insignificant.

THE CULTURES.—Each culture consisted of 100 seedlings in 250 cc. of standard nutrient solution contained in a 300-cc. flask.   The standard nutrient solution was SHIVE's solution R5C2 (39), calculated to have an
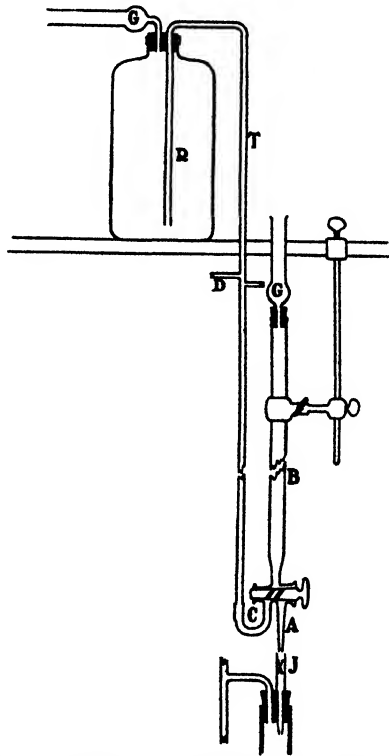
FIG. 2.   Diagram showing arrangement of burette and reservoir for charging the CO_2-absorption apparatus.   For description see text.

osmotic value of 0.175 atmosphere.   Each liter contained 0.1228 gm. of $Ca(NO_3)_2$, 0.2452 gm. of KH_2PO_4 and 0.3698 gm. of $MgSO_4$; no iron was added.   Eight liters of stock nutrient solution were prepared, 10 times as concentrated as was required.   The salts used were from unopened bottles originally prepared in connection with the cooperative experiments planned by the Division of Biology and Agriculture of the National Research Council (27), by the Powers-Weightman-Rosengarten Co.   The concentrated stock nutrient solution was prepared by dissolving the three salts together, to form

a measured volume of solution, rather than by mixing separate standardized single-salt solutions. No precipitate appeared.

When a set of five cultures was to be started the requisite volume of solution was made up by diluting 125 cc. of the stock solution to 1250 cc., 250 cc. of which was placed in each of the five culture flasks, which were numbered and stoppered and then distributed in the five temperature chambers, where each flask and its contents soon came to the temperature of the chamber. Then the 1,000 germinated seeds that had been for 42 hours in water in the germination flask in the middle chamber (19.5°) were spread out in a glass pan, from which they were transferred one by one (with bone-tipped forceps) to the culture flasks, which were removed from their chambers and remained at room temperature only for the 20 minutes required for selection and transfer. In order that the five 100-seedling lots might be as nearly alike as possible, that every lot might contain about the same proportions of somewhat larger, somewhat smaller seeds, etc. (although, as has been said, the 500 seedlings selected for a set of cultures were very nearly alike), the five lots were not selected consecutively; instead of that, 10 or 20 seedlings were transferred to one flask, then a like number to another flask and so on till every flask had received its 10 or 20 seedlings, after which this procedure was repeated in rotation, till every flask had its full quota of 100 seedlings. As soon as the transfer had been completed each flask was returned to the chamber in which it and its solution had previously been warmed or cooled, being immediately placed in connection with the gas line (with stopper set firmly in place and flask clamp tightened to insure a permanent seal) and the flow of gas mixture began.

At the close of a series of experiments (i.e., immediately after the titration for the fifth test interval) the culture flasks were removed from the apparatus and their seedlings were examined with respect to the growth that had occurred. The seedlings were then discarded and the flasks were scrubbed with hot water, rinsed with distilled water and thoroughly dried, being then ready for another series of experiments. No special procedure was adopted for the sterilization of flasks and nutrient solution, nor were the wheat seeds disinfected before being placed in the germination flask. There was no evidence of growth of fungi or bacteria in any of the cultures, even at the higher temperatures.

Changes in the culture solution in the experiment period were ignored, as has been said. That such changes must have occurred, because of absorption and excretion, was indicated by a few brief studies with seedlings in nutrient solutions in which other seedlings had previously been grown for a 24-hour period, but these changes were found to be slight, probably because of the large volume of solution used for each culture and the short duration of the experiment period.

MEASUREMENTS OF CO_2 PRODUCTION.—Measurements of CO_2 production were begun 2 hours after each series of culture flasks received their seedlings, or approximately 1.5 hour after the cultures were placed under the experimental conditions. At the end of each test interval the total production of CO_2 for that interval (the amount of CO_2 that had been absorbed in the absorption apparatus during the interval) was ascertained by titration. About half an hour was required to make the five titrations, which were made always in the same order from the lowest to the highest temperature, so that the intervals were very nearly the same for the different experiments in a series. The deviation from the stated time of observation was usually less than 15 minutes; in a few cases, however, it was greater, sometimes as much as 45 minutes. The actual length of the time interval referred to by each titration record was recorded in every instance, however, and the mean hourly rate of CO_2 production was computed. These mean hourly rates for the five test intervals of each experiment are the numerical data on CO_2 production with which we shall deal when the results of these experiments are presented farther on.

OBSERVATIONS ON GROWTH.—At the end of each experiment, 48 hours after the seedlings had been placed in their experimental environment, the seedlings were removed from the culture solutions and measurements of growth were made. Although the roots elongated more rapidly than the shoots their measurement presented so many difficulties (especially because the number of roots per seedling was variable) that growth records were based on shoot elongation alone. Shoot elongation also was variable, especially at the higher temperatures, and many seedlings made so little growth in the experiment period that actual measurement was not practicable. After considerable study the following procedure was adopted for securing numerical indices of shoot elongation. All shoots that could be measured with satisfactory accuracy were measured to the nearest 0.2 millimeter, using a millimeter scale, and the average of the ten longest ones in an experiment was taken as the measure of the growth rate for that experiment. Of course these indices of growth rate are not precise, but they are sufficiently consistent to be useful, as will be seen later.

THE COMPUTATION OF AVERAGES.—The total amount of CO_2 collected during an interval was first expressed in terms of the equivalent volume of standard acid solution, in cubic centimeters, and this value was then multiplied by the standard factor 2.49, for 1 cc. of standard acid solution had been shown to be equivalent to 2.49 mg. of CO_2. The resulting product was the number of milligrams of CO_2 produced by the given culture in the given interval. This product was next divided by the length of the corresponding interval, expressed in hours, to give the mean hourly rate of CO_2 production for the interval. Five of these mean hourly rates were

thus secured for each experiment and 25 for each experiment series. In one or two instances where the number of seedlings per culture was not 100 the mean rates were all computed to represent 100 seedlings, which they did actually represent in most cases.

A preliminary, point-to-point graph was drawn to represent the five mean rates for each interval, as these differed from temperature to temperature in the same experiment series, one graph for the first interval, another for the second, etc. The ordinates of these preliminary graphs were the mean hourly rates of CO_2 production and the abscissas represented the corresponding temperatures, derived from thermograph or thermometer records. Each experiment series thus gave five graphs, one for each of the consecutive test intervals, and each group of graphs represented one of the 12 different oxygen-nitrogen mixtures used. Since the actual temperature means were not always exactly the standard maintained temperatures that they represented, the ordinates of each graph were corrected where necessary, to correspond more closely to the requisite temperatures, by simply measuring the actual ordinates for those temperatures ($10°$, $15°$, $20°$, $25°$, and $30°$) and employing the resulting values instead of the actual mean rates from which the graph had been constructed. By these procedures a mean hourly rate of CO_2 production by 100 seedlings was obtained for each of the five required temperatures and for each of the five test intervals in every experiment series, some slight discrepancies being thus avoided while all series were brought into conformity with regard to the five temperature values. Because every experiment series was repeated at lest once there were two or more mean hourly rates of CO_2 production for each of the 60 different combinations of temperature and oxygen pressure for each interval and these were finally averaged for each combination.

For the entire experiment period the total amounts of CO_2 produced with each combination of temperature and oxygen pressure were ascertained for each experiment, and these totals were finally averaged for each group of like experiments. Sixty average totals were thus secured, one for each combination of temperature and oxygen pressure used.

Results and discussion

GENERAL NATURE OF THE RESULTS

The main results of this study will now be presented, with notes on their interpretation. It is to be borne in mind that the conclusions reached are applicable only to the specific sets of internal and external conditions that prevailed in the tests. The data presented are to be studied to bring out any relations that may appear among five kinds of variables, always

with reference to the conditions of the internal and external background of the experiments, which have been described. These variables are: (1) maintained temperature, (2) partial pressure of oxygen, (3) relative stage in the progress of seedling development (indicated in each experiment by the interval number), (4) rate of CO_2 production and (5) rate of shoot elongation. Expressed in another way, the results are to be considered with reference to the manner in which the rates of CO_2 production and of shoot elongation differ, from interval to interval in the same experiment, and from experiment to experiment in relation to the several combinations of maintained temperature and maintained oxygen pressure. There are to be considered 12 different partial pressures of oxygen (the total gas pressure being always 1 atmosphere) for each of the five different maintained temperatures, and each of the 60 different combinations of temperature and oxygen pressure is to be considered with regard to each of the five consecutive test intervals of each experiment.

No Evidence of Aging of Seeds

The experiment series with partial pressure of oxygen about like that of ordinary air (20 per cent.) was carried out seven times, as chronologically numbered series 1, 2, 3, 4, 23, 37 and 38. (Series 5–22 and 24–36 had other oxygen pressures.) The first four series were carried out between December 10 and December 18, 1928, series 23 was carried out in February, 1929, and the last two were carried out between March 20 and March 24, 1929. The actual partial pressures of oxygen for these series were: series 1, 2 and 3, 21.3 per cent.; series 4, 20.4 per cent.; series 23, 20.0 per cent.; series 37 and 38, 18.9 per cent.; but these differences are not large enough to be significant. A study of the results of these series (brought together in table I) fails to reveal any consistent suggestion that the seedlings of series 1 or 2 may have been initially different from those of series 37 or 38 and leads to the conclusion that the stock of seed used in this study did not alter significantly during the progress of the work. It is also clear from the value of table I that the standard germination technique was not significantly altered as the work went on. These are considerations of the utmost importance in studies of this kind.

CO_2 Production in the Several Observation Intervals and in the Entire Experiment Period

THE TABULATED VALUES.—The 60 average rates of CO_2 production for each of the five intervals are represented in table II. The five horizontal subdivisions of the table represent the five test intervals, which are numbered consecutively and designated in the following manner: 1st interval,

TABLE I

COMPARISON OF RESULTS FROM EXPERIMENT SERIES NEAR BEGINNING AND NEAR END OF STUDY, WITH OXYGEN PRESSURE ABOUT 20 PER CENT.

EXPERIMENT SERIES NO.	DATES OF EXPERIMENT	TEST INTERVAL	MEAN HOURLY RATE OF CO_2 PRODUCTION (100 SEEDLINGS) FOR TEMPERATURE OF				
			10° C.	15° C.	20° C.	25° C.	30° C.
			mg.	mg.	mg.	mg.	mg.
1	Dec. 12–14 1928	1	0.33	0.55	0.70	0.88	1.25
		2	0.34	0.62	0.77	1.14	1.88
		3	0.36	0.77	1.07	1.37	2.13
		4	0.39	0.94	1.18	1.76	2.78
		5	0.46	1.12	1.50	2.26	3.63
2	Dec. 14–16 1928	1		0.61	0.83	0.87	1.37
		2		0.60	0.85	1.15	1.93
		3	0.37	0.76	1.03	1.35	2.38
		4	0.47	0.93	1.29	1.75	3.27
		5	0.49	1.08	1.69	2.34	3.82
3	Dec. 16–18 1928	1	0.36	0.78	0.95	1.10	1.39
		2	0.42	0.77	0.93	1.40	1.78
		3	0.52	0.83	1.09	1.59	2.47
		4	0.66	1.01	1.31	2.04	3.57
		5	0.73	1.19	1.58	2.72	4.50
4	Dec. 18–20 1928	1	0.40	0.74	0.84	0.95	1.49
		2	0.39	0.72	0.87	1.08	1.81
		3	0.51	0.84	1.06	1.42	2.37
		4	0.58	0.98	1.41	2.07	3.49
		5	0.68	1.22	1.69	2.49	4.03
23	Feb. 12 1929	1	0.35	0.60	0.77	1.02	1.19
		2	0.42	0.62	0.78	1.12	1.63
		3	0.45	0.73	1.02	1.61	2.32
37	Mar. 20–22 1929	1	0.44	0.62	0.82	0.96	1.23
		2	0.46	0.62	0.81	1.07	1.64
		3	0.48	0.71	1.04	1.40	2.14
		4	0.54	0.91	1.29	1.77	3.01
		5	0.63	1.22	1.71	2.33	4.00
38	Mar. 22–24 1929	1	0.43	0.52	0.80	0.87	1.20
		2	0.41	0.52	0.89	1.18	1.64
		3	0.47	0.73	1.16	1.47	2.14
		4	0.54	0.88	1.50	1.98	3.21
		5	0.68	1.09	1.87	2.51	

Note.—Experiment series 23 was continued for three intervals only. The two blanks for series 2 and one for series 38 indicate that the data were lost.

the four hours following the close of the adjustment period (*i.e.* the 3rd to the 6th hour, inclusive, after transfer of seedlings to culture flask) ; 2nd interval, the next 6 hours (7th to 12th hour of the experimental period) ; 3rd interval, the next 12 hours (13th to 24th hour) ; 4th interval, the next

TABLE II

AVERAGE RATES OF CO₂ PRODUCTION BY 100 SEEDLINGS FOR EACH TESTED COMBINATION OF TEMPERATURE AND OXYGEN PRESSURE IN EACH OBSERVATION INTERVAL

INTERVALS	TEMPERATURE (deg. C.)	AVERAGE MEAN HOURLY RATE OF CO₂ PRODUCTION WITH OXYGEN PRESSURE OF											
		0.6 PER CENT.	3.1 PER CENT.	6.3 PER CENT.	9.8 PER CENT.	16.0 PER CENT.	20.0 PER CENT.	30.0 PER CENT.	50.0 PER CENT.	75.0 PER CENT.	90.0 PER CENT.	95.0 PER CENT.	98.3 PER CENT.
		mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
1ST TEST INTERVAL (3RD TO 6TH HOUR, INCLUSIVE)	10	0.36	0.38	0.38	0.42	0.44	0.39	0.44	0.45	0.47	0.46	0.46	0.46
	15	0.46	0.55	0.61	0.56	0.58	0.63	0.71	0.74	0.82	0.84	0.75	0.71
	20	0.63	0.73	0.73	0.86	0.76	0.82	0.83	0.94	1.10	1.17	1.02	0.98
	25	0.74	0.88	0.93	1.02	0.97	0.95	1.05	1.20	1.38	1.50	1.29	1.23
	30	0.96	1.03	1.16	1.21	1.23	1.30	1.34	1.60	1.91	2.08	1.87	1.55
2ND TEST INTERVAL (7TH TO 12TH HOUR, INCLUSIVE)	10	0.26	0.33	0.31	0.34	0.41	0.41	0.54	0.56	0.59	0.64	0.52	0.53
	15	0.41	0.52	0.56	0.54	0.56	0.64	0.72	0.85	0.98	1.04	0.93	0.92
	20	0.61	0.73	0.77	0.80	0.83	0.84	0.94	1.05	1.37	1.57	1.38	1.36
	25	0.79	1.00	1.14	1.37	1.20	1.16	1.24	1.44	1.81	2.04	1.74	1.80
	30	1.11	1.32	1.72	1.86	1.75	1.76	1.69	2.02	2.56	2.88	2.55	2.42
3RD TEST INTERVAL (13TH TO 24TH HOUR, INCLUSIVE)	10	0.27	0.30	0.34	0.33	0.44	0.45	0.59	0.69	0.75	0.73	0.66	0.63
	15	0.46	0.62	0.61	0.52	0.62	0.78	0.83	0.97	1.30	1.42	1.55	1.25
	20	0.66	1.00	1.11	1.06	1.08	1.07	1.21	1.43	1.98	2.41	2.20	2.24
	25	0.93	1.52	1.81	1.95	1.74	1.46	1.67	1.93	2.80	3.52	3.16	3.18
	30	1.38	1.97	2.29	2.36	2.62	2.28	2.25	2.88	3.88	4.65	4.54	4.07
4TH TEST INTERVAL (25TH TO 36TH HOUR, INCLUSIVE)	10	0.28	0.35	0.37	0.36	0.51	0.53	0.70	0.86	0.82	0.89	0.76	0.77
	15	0.47	0.88	0.82	0.70	0.75	0.94	1.04	1.25	1.66	1.91	2.04	1.52
	20	0.70	1.30	1.37	1.18	1.17	1.33	1.46	1.88	2.82	3.49	3.60	3.54
	25	1.03	1.85	2.06	2.31	2.21	1.90	2.00	2.75	3.99	4.56	5.14	4.65
	30	1.41	2.38	2.56	3.19	3.76	3.24	3.11	4.63	5.22	5.93	6.13	5.47
5TH TEST INTERVAL (37TH TO 48TH HOUR, INCLUSIVE)	10	0.29	0.44	0.45	0.37	0.60	0.61	0.83	1.05	1.06	1.15	1.11	1.04
	15	0.50	1.01	1.02	0.87	0.91	1.15	1.34	1.71	2.41	2.90	3.34	2.55
	20	0.80	1.41	1.54	1.55	1.43	1.67	1.83	2.58	3.81	4.70	5.01	4.86
	25	1.23	2.14	2.30	2.93	2.97	2.45	2.75	3.87	4.94	5.66	6.30	5.72
	30	1.48	2.70	3.08	3.72	4.66	4.00	4.34	5.81	6.14	6.67	6.87	6.38
NUMBER OF EXPERIMENTS		3	2	2	2	4	7	3	2	4	3	3	3

12 hours (24th to 36th hour); 5th interval, the last 12 hours of the experiment period (37th to 48th hour). Each line of the table shows values for a single temperature and each column shows values for a single oxygen pressure. The number of experiments on which the values of each column are based is shown at the bottom of the column. The probable errors of the averages were computed in a large number of cases by BESSEL's (30) formula, and they were found in most cases to be less than 4 per cent. of the average itself; in a very few cases the probable error was about 6 per cent.·of the average. It is probably safe to suppose that the lowest values given in table II represent the facts within plus or minus 0.02 or 0.03 mg. and that the highest values are thus representative within plus or minus 0.4 or 0.5 mg.

From the averages given in table II, the most obvious facts to be noted are that these experiments gave rates of CO_2 production varying in magnitude from below 0.3 mg. to nearly 7.0 mg. per hour, for 100 seedlings, and that the magnitudes of the rates are clearly related to the temperature-oxygen combination used and to the developmental phase of the plantlets.

PROGRESSIVE INCREASE IN CO_2 PRODUCTION THROUGHOUT THE EXPERIMENT PERIOD.—It is generally evident from table II that the rate of CO_2 production for any combination of temperature and oxygen pressure became progressively greater with time; the rate for a later interval generally more or less exceeds the corresponding rate for any earlier interval in the same experiment. This would be expected, for the rate here considered should generally be greater as development of the plantlets proceeds through its earlier phases. The developmental progress of CO_2 production must have been very slow at the inception of germination and this progress in the experiment period fails to be indicated or was only slightly marked for combinations of low temperature and low oxygen pressure. This progress is generally well shown, however, for combinations of low temperatures and high oxygen pressure and for combinations of high temperatures and any oxygen pressure tested, being most pronounced for the combination of 30° and 90 or 95 per cent. of oxygen in the gas mixture.

The time graphs of figures 3 and 4 are representative samples of a complete series of graphs representing all the data in table II. Figure 3 shows the five time graphs, one for each of the temperatures tested, for each of six representative oxygen pressures; namely, 9.8, 20, 50, 75, 90 and 98.3 per cent. Each section of the figure shows the numerical data for CO_2 production (ordinates) for each of the observation intervals (abscissae), there being five graphs in each case, one for each of the maintained temperatures used. The temperature represented is shown at the right-hand end of each graph. Each of these single graphs thus shows the time march of CO_2 production from the first interval (3rd to 6th hour after the
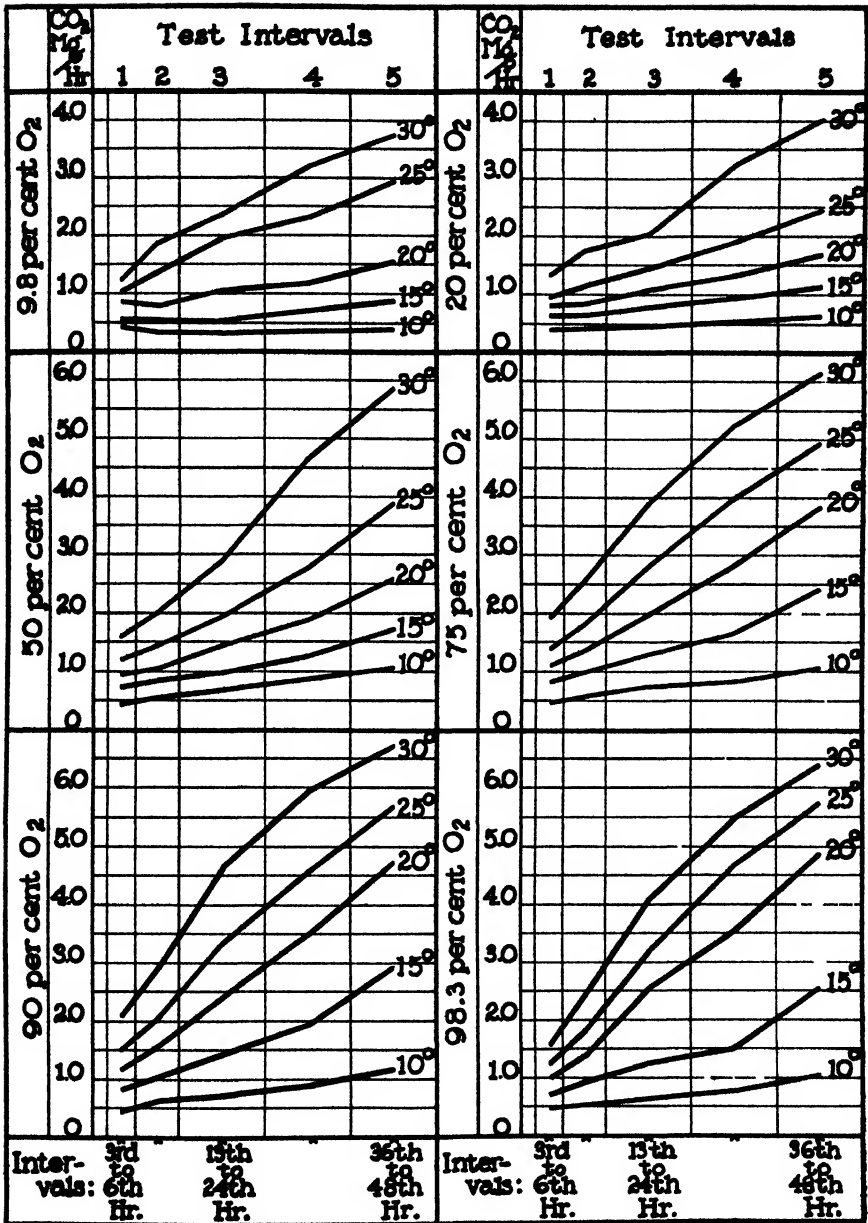
Fig. 3. Representative time graphs showing the march of CO_2 production during the experiment period for each temperature as related to oxygen pressure. These are arranged in groups, each group representing a different oxygen percentage.

start of the experiment) to the fifth interval (37th to 48th hour). In each of the time graphs (including those of figure 4 as well as those of figure 3) the abscissa for any point represents the middle of the observation interval in question; for example, data for the second interval (7th to 12th hour of the period) are plotted as referring to the end of the 9th hour.

In no section of the series of graphs represented by figure 3 is there any crossing or coincidence of the graphs; with every oxygen pressure tested the five-degree temperature intervals were amply great to insure perfectly distinct graphs of this sort and it is apparent that much narrower temperature intervals might have been employed without the introduction of confusion due to unexplained variability among the lots of seedlings. Of course the five graphs for any oxygen pressure approach one another for the shorter abscissae, since the rates of CO_2 production must all have approached zero at the beginning of the germination period, no matter what combination of temperature and oxygen pressure was used.

The graphs of figure 4 are in part the same graphs as are shown in figure 3. In figure 3 there is for each one of six representative *oxygen pressures* a group of five time graphs (*for the five temperatures*) and each section of the figure represents *a single oxygen pressure*. In figure 4, on the other hand, there is for each of the five *temperatures* a group of four or five time graphs (*for representative oxygen presures*) and each section of this figure represents *a single temperature*. By the first arrangement the graphs are grouped according to oxygen presure while they are grouped according to temperature by the second arrangement. In figure 4 both the lowest and the highest graph are shown for each temperature, with one or two intervening graphs for representative oxygen pressures between the pressure for the lowest and that for the highest graph. These are all full lines. For 10° they are for oxygen pressures of 0.6, 20 and 90 per cent. and for the other temperatures they are for 0.6, 20, 50 and 95 per cent. (In the few instances where the same graph is not highest with respect to all intervals the graph shown as highest is the one that is highest for the fifth interval.) In addition, the graph for the oxygen pressure of 98.3 per cent. is shown in every case, its line being broken to distinguish it more readily from the other graphs.

Some temperature-oxygen combinations failed to show a regular increase in the rate of CO_2 production from interval to interval and these exceptions are of special interest. They are listed here for the sake of inspection. Each combination is shown, in the following lists, by its temperature and its oxygen-percentage value separated by a comma, and the value in parenthesis is the difference (in hundredths of a milligram) between the two average rates considered. For the following thirteen combinations of temperature and oxygen pressure the average for the second interval

does not exceed that for the first: 10°, 0.6 (10); 10°, 3.1 (5); 10°, 6.3 (7); 10°, 9.8 (8); 10°, 16 (3); 15°, 0.6 (5); 15°, 3.1 (3); 15°, 6.3 (5); 15°, 9.8 (2); 15°, 16 (2); 20°, 0.6 (2); 20°, 3.1 (0); 20°, 9.8 (6). For the following eight combinations the average for the third interval does not exceed that for the first: 10°, 0.6 (9); 10°, 3.1 (8); 10°, 6.3 (4); 10°, 9.8 (9);

FIG. 4. Representative time graphs showing the march of CO_2 production during the experiment period for each oxygen pressure as related to temperature. These are arranged in groups corresponding to the several temperatures, each group representing a different temperature.

10°, 16 (0); 15°, 0.6 (0); 15°, 6.3 (0); 15°, 9.8 (4). For the following four combinations the rate for the fourth interval does not exceed that for the first: 10°, 0.6 (8); 10°, 3.1 (3); 10°, 6.3 (1); 10°, 9.8 (6). Only for
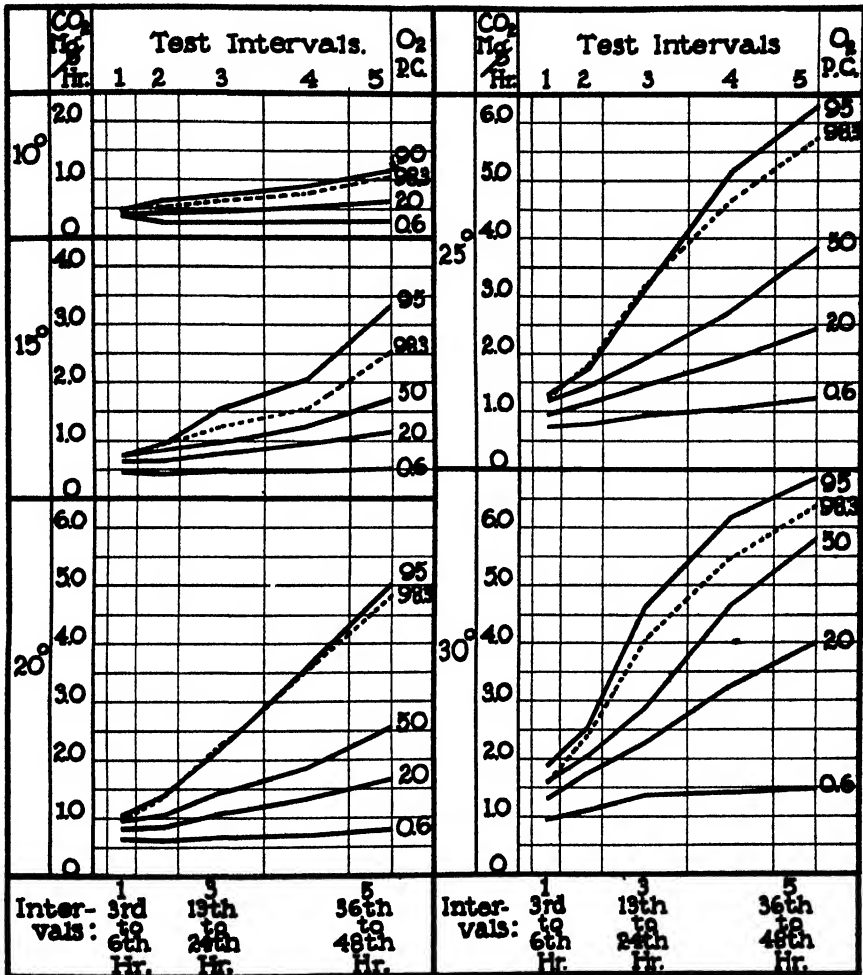
the combination of 10° and 0.6 per cent. does the rate for the fifth interval fail to exceed that for the first. These exceptions are all confined to temperatures of 20° or lower and to oxygen pressures of 16 per cent. or lower and they correspond to environmental complexes that gave relatively low rates of CO_2 production in any interval. In some of these instances the exceptions may be due to possible errors in observation, which were of course relatively larger for these lowest rates, but many of them appear to be significant, suggesting that the effect of the environmental change from germination flask to culture flask (or the lagging influence of the environmental conditions of the germination period extending into the experimental period) required more or less of the experiment period in which to disappear. It is possible that the effect of the environmental change itself may have been important in determining the higher rate of CO_2 production in the earlier test intervals, in the cases where this occurred. It has been mentioned in various connections in the literature that a sudden change in the environment may result in a stimulation or retardation of activity, either of which may be opposite in tendency from the direct effect of the conditions prevailing after the change. A recent notable example of this is contained in a paper by PARIJA (34), in which the graphs illustrate pronounced temporary increases in CO_2 production by apples when they were subjected to a change from one atmospheric environment to another with a lower oxygen percentage, and a temporary reduction in CO_2 production for an environmental change of the opposite description, for changes between several different oxygen percentages, including ordinary air, pure oxygen, pure nitrogen, and percentages of oxygen from 3 to 9 at intervals of 2 per cent.

In this connection we may neglect the data for the first interval and consider the period of transfer and adjustment as of 8 rather than 2 hours. When that is done an examination of table II shows only three exceptions of the sort here considered; only for the three combinations, 10°, 3.1 (3); 10°, 9.8 (1) and 15°, 9.8 (2), were the rates for the second interval greater than those for the third and these exceptions are negligible. Rates for the second interval were in all cases smaller than the corresponding rates for the fourth or fifth.

Because of the progressive increase in the rate of CO_2 production throughout the period, the time graphs generally slope upward from low ordinates at the left to higher ones at the right. The mean rate of upward slope is usually greater with higher temperature than with lower, for any oxygen pressure. It is usually greater with higher oxygen pressure than with lower, for any temperature, but generally there is indicated an optimal oxygen pressure below 98.3 per cent. In no case does a graph bend downward at the right, which indicates that no maximum rate of CO_2 production

was attained in the experiment period with any of these complexes of conditions. In some cases the graphs beyond the second interval. are nearly rectilinear (*e.g.*, that for 25° and 20 per cent.) in other cases they are somewhat concave upward in the region of the later intervals, and in still other cases they are somewhat convex upward in that region. Of course upward convexity means a decreasing acceleration of the rate of CO_2 production and suggests that a maximum in that rate may have been approached. This last suggestion applies especially to the graphs for 30°, a temperature that must be regarded as surely supra-optimal for health in such seedlings as these, for it is likely that pathological conditions supervened before the close of the experiment period in all cultures maintained at 30°.

In each section of figure 4 the upward slope of the time graphs is seen to be generally steeper for any temperature as the oxygen pressure is greater, until the highest graph for the given temperature is reached, but the graph for a pressure of 98.3 per cent. (broken line) regularly lies below the highest graph. The highest oxygen pressure (nearly pure oxygen) gave generally lower average rates of CO_2 production for any temperature and observation interval than did the slightly lower pressure that gave the corresponding highest rate for that temperature and interval. In the whole series of time graphs represented by figure 4 the only exceptions to this rule are for 10° in the first interval. The rates for 10° in the first interval are low and alike for 90, 95 and 98.3 per cent. For 25° in the second and third intervals the graph for 98.3 per cent. lies slightly above that for 95 per cent., but in these two instances the highest rates were given by an oxygen pressure of 90 per cent. and reference to table II shows that these two ordinates of the graph for 98.3 are both much smaller than the corresponding values for 90 per cent.

SPECIAL STUDY OF THE COMBINATION OF 20° AND THE OXYGEN PRESSURE OF 20 PER CENT. IN THE SEVERAL INTERVALS.—Special attention may be called to the behavior of the seedlings subjected to the maintained combination of 20° and 20 per cent. The values for this combination of a commonly experienced temperature and ordinary air may be considered as fairly representative of many germination tests and of what occurs when such seed as was used in this study is allowed to germinate in field plantings. These seedlings had been transferred from water to the standard nutrient solution at the beginning of the experiment but their temperature and oxygen pressure had not been significantly different during the experiment period from what these had been during the germination period. For the youngest development phase studied (1st interval, when shoots were only about 0.5 mm. long and roots were barely showing through the coleorhiza) the average mean rate of CO_2 production was 0.82 mg. per hour per 100

seedlings. The rate increased as time went on (being 0.84 mg. for the 2nd interval, 1.07 mg. for the 3rd interval, 1.33 mg. for the 4th interval) until it had a value of 1.67 mg. for the last interval, when the seedlings had shoots about 5 mm. long and roots about 20 mm. long. It is remarkable that this series of values, from 0.84 for the 2nd interval (say 9 hours after the beginning of the experiment), to 1.67 for the 5th interval (say 42 hours after the beginning of the experiment), shows an increase that is almost proportional to time, at the rate of about 0.025 mg. per hour.

RATES IN THE SECOND INTERVAL.—Because some of the rates of CO_2 production in the first interval appear to show influence of the environmental change that occurred when the cultures were started, the earliest interval which is practically without such inconsistencies is the second, the data for which may be taken to represent these seedlings at a very early developmental stage. These data are shown in the second horizontal section of table II and by the graphs of figures 5 and 6.

The chart of figure 5 was planned to show how these very young seedlings (all of the same age with respect to time) exhibited different rates of CO_2
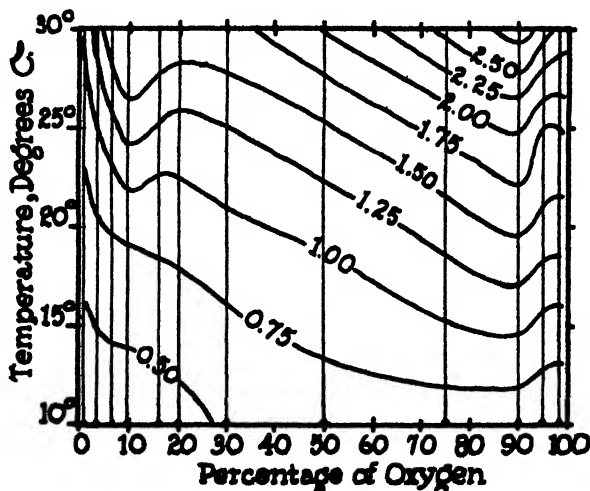
FIG. 5. Diagram showing by contours or isopleths, the relations between rate of CO_2 production (by 100 seedlings) and maintained temperature-oxygen combinations for the second test interval. Points of intersection of the vertical and horizontal lines represent the values given in table II. For all points on any contour the average rate of CO_2 production is the same, its value being indicated by the number on the contour (0.50 to 2.50).

production according to the particular maintained combinations of temperature and oxygen pressure to which they had been subjected since the cultures were started. This chart represents a solid diagram in which "east-west" distances represent progressively larger partial pressures of

oxygen, "south-north" distances represent progressively higher maintained temperatures, and altitudes (as shown by the values on the isopleths or contours) represent hourly rates of CO_2 production. Isopleths are shown for each rate from 0.50 mg. to 2.75 mg., by intervals of 0.25 mg. A glance at this diagram will show what average hourly rate corresponds to any tested combination of temperature and oxygen pressure, or what combinations of these factors correspond to any particular average rate, for the second observation interval. It is notable that the same rate may correspond to many different combinations of temperature and oxygen pressure. For example, the rate of 1.0 mg. per hour per 100 seedlings in the second test interval coresponds to combinations of about: 14.5° and 86 per cent., 17.5° and 58 per cent., 20° and 38 per cent., 22.75° and 18 per cent., 22° and 10 per cent., 28.5° and 0.3 per cent., etc., etc. This manner of presenting the numerical data emphasizes the generalization that neither temperature alone nor oxygen pressure alone is to be considered as the determining condition for any rate of CO_2 production; both of these factors must be considered and there is a broad range of combinations of them that correspond to the same rate.

It is to be noted that the isopleths of figure 5 are in general closer together (*i.e.*, the slope of the surface represented by the diagram is steeper) in the region of combinations of the higher values of the two experimental variables than in the region of combinations of the lower values. In other words, these seedlings were less sensitive, as far as the rate of CO_2 production is concerned, to differences between combinations of high temperature and high oxygen pressure than to differences between combinations of low temperature and low oxygen pressure.

It is interesting to observe also that, for oxygen pressures between about 20 per cent. and about 90 per cent., the temperature corresponding to any average hourly rate of CO_2 production is generally lower as the oxygen pressure is higher, the oxygen pressure corresponding to any rate is lower as the temperature is higher, and the relation between temperature and oxygen pressure for any average rate above about 1 mg. per hour is nearly a linear one and about the same for all these rates. Within the region of the diagram here considered (between oxygen pressure of about 20 per cent. and about 90 per cent.) the percentage value of the oxygen pressure giving any average rate of CO_2 production above about 1 mg. per hour is decreased by about 15 for each temperature increase of about 2°. Rates of CO_2 production below about 1 mg. per hour show contours with progressively lower slopes in the region of approximate linear relation between temperature and oxygen pressure, the left margin of this region being shifted to the right. The characteristic forms of the contours for the region of oxygen pressures below about 20 per cent. and for the region

of pressures above about 90 per cent. bring out other modifications which will be referred to below. Within the range of oxygen pressure between about 20 and about 90 per cent. the rate of CO_2 production for any pressure is shown to increase rather regularly with increase in temperature, somewhat more rapidly in the higher than in the lower temperature ranges. Also, the rate of CO_2 production for any temperature is shown to increase rather regularly with increase in oxygen pressure within the specified range, somewhat more rapidly for higher than for lower temperatures.

Other diagrams similar to that of figure 5, for the data of the 3rd, 4th and 5th intervals and for the experiment period as a whole are just as interesting and instructive as the one here shown. They may be readily constructed from the data given in table II.

Figure 6 presents plane graphs of the data on CO_2 production in the second interval. Ordinates represent average hourly rates of CO_2 production and abscissae represent percentages of oxygen in the gas mixture.
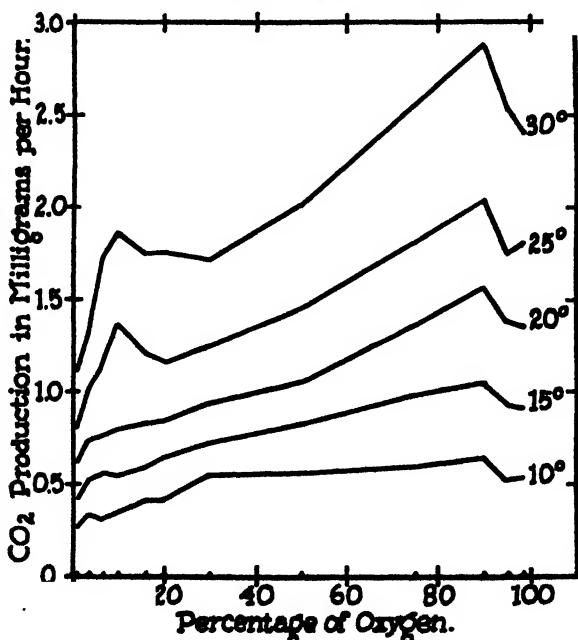
FIG. 6. Graphs of CO_2 production in the second observation interval, as related to oxygen pressure and to temperature. Each graph represents a different temperature.

There are five graphs in each group, one for each temperature tested.

Two graph maxima are clearly shown for 25° and 30° and suggested for 10°, 15° and 20°. For 25° and 30° the first graph maximum corresponds to a pressure of 9.8 and in all cases the second graph maximum

corresponds to a pressure of 90 per cent. Between these two graph maxima there is indicated a minimum region, the minimum itself corresponding to 20 per cent. for 25° and to 16–30 per cent. for 30°.

The existence of two graph maxima implies two optimal oxygen pressures for the given temperature, the first (lower) optimal pressure being 9.8 per cent. for 25 and 30° and the second (higher) optimal pressure being 90 per cent. The second or main optimum of pressure is clearly shown for all temperatures tested in the second interval, but the first optimum is only suggested for the three lower temperatures, for which it appears to be progressively lower with lower temperature. The double optimum of oxygen pressure is also indicated by the contours of figure 5; the graphs of figure 6 are of course simply "east-west" profiles of the area represented by figure 5. More attention will be given to this double optimum in connection with the discussion of the rates for the entire period, in the next following section.

Another aspect of the complex set of relations shown by figures 5 and 6, for the second observation interval, may be illustrated by noting that although the rate of CO_2 production is shown to be generally higher with higher temperature and also with higher oxygen percentage, within the ranges studied, yet the two influences are not generally equally effective. The temperature influence is most effective with an oxygen pressure of about 90 per cent. and least effective with a pressure of 0.6 per cent., but it is more effective with 10 than with 20 per cent. and less effective with 98.3 than with 90 per cent. The plantlets were able to produce CO_2 most rapidly, in this second interval, when the temperature effectiveness was high; that is, when a given increase in temperature gave a large increase in CO_2 production.

The rate of CO_2 production for any temperature in the second interval is shown by the plane graphs to have been nearly proportional to the oxygen pressure for a range of pressure between about 20 or 30 per cent. and about 90 per cent., the upward slope of the nearly rectilinear graphs for that range being progressively steeper with higher temperature. A similar observation has been noted from the isopleths of figure 5.

CO_2 PRODUCTION IN THE ENTIRE EXPERIMENT PERIOD.—The data for total CO_2 production in the whole experiment period are given in table III and are represented by the graphs of the first part of figure 7. The arrangement of these graphs is like that of the graphs in figure 6, ordinates being the average rates of CO_2 production while abscissae are oxygen pressures, with a separate graph for each of the five temperatures. The units shown at the left of the figure are for average hourly rates for the whole period; *i.e.*, each total value given in table III has been divided by 46 (the number of hours in the experiment period) to give the corresponding ordinate shown in the graphs.

TABLE III

TOTAL CO_2 PRODUCTION BY 100 SEEDLINGS FOR EACH COMBINATION OF TEMPERATURE AND OXYGEN PRESSURE IN THE ENTIRE EXPERIMENT PERIOD

TEMP-ERATURE	AMOUNT OF CO_2 PRODUCED IN 46 HOURS (BY 100 SEEDLINGS) WITH OXYGEN PRESSURE OF											
	0.6 PER CENT.	3.1 PER CENT.	6.3 PER CENT.	9.8 PER CENT.	16.0 PER CENT.	20.0 PER CENT.	30.0 PER CENT.	50.0 PER CENT.	75.0 PER CENT.	90.0 PER CENT.	95.0 PER CENT.	98.3 PER CENT.
Deg. C.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
10	12.8	16.6	17.0	16.6	22.5	23.0	30.3	36.3	36.8	39.1	35.4	34.5
15	21.6	37.2	35.4	30.3	33.1	40.8	45.5	55.2	73.6	82.3	91.5	72.2
20	31.3	51.9	55.7	52.4	51.9	57.1	63.0	80.4	115.8	141.2	142.1	139.8
25	46.0	75.4	84.6	98.4	93.8	80.4	88.3	115.8	157.3	180.2	190.8	178.3
30	61.6	96.6	110.3	127.3	144.3	130.2	131.4	178.3	205.1	232.6	228.6	211.4
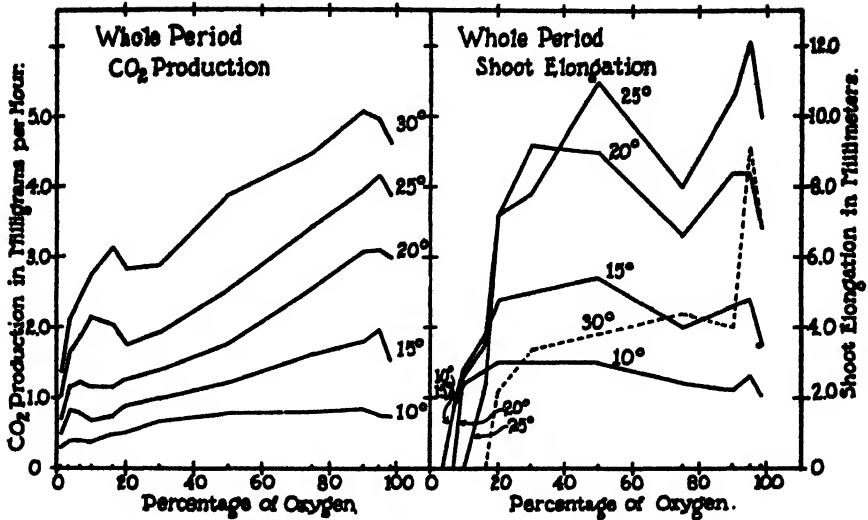
FIG. 7. Graphs of CO_2 production and of shoot elongation in the entire experiment period, as related to oxygen pressure and to temperature. Each graph represents a different temperature.

The graphs for the entire period are very similar to those for the second interval. The two graph maxima shown for the second interval are clearly shown here for 15°, 20°, 25° and 30° and are suggested for 10°. The portion of each graph between the subordinate minimum and the second maximum is nearly rectilinear, with the slope progressively steeper as the temperature is higher, as is true of the graphs for the higher temperatures in the second interval. The first graph maximum corresponds to higher oxygen pressure with higher temperatures; for 10° and 15° it is indicated as corresponding to 3.1 per cent., for 20° it corresponds to 9.8 per cent., and for 30° it corresponds to 16 per cent. These pressure values are those of the first or subordinate optimal pressure. The second graph maximum corresponds to a pressure of 90 or 95 per cent. for all temperatures and this slight variability appears not to be related to temperature. The abscissa of the second graph maximum is of course the main or second optimal oxygen pressure in each instance.

It is specially remarkable that the main optimal oxygen pressure is shown as below 98.3 per cent. for every temperature tested in every interval and in the whole period; this highest pressure tested was surely supraoptimal in every instance. Reference to table II shows that the main optimal oxygen pressure (the average rates for which are there shown in bold-face type) was either 90 or 95 per cent. in every case excepting 10° in the first and third intervals, for which it is shown as 75 per cent.

The subordinate graph minimum for the graphs of the whole period generally corresponds, like the first maximum, to an oxygen pressure that is somewhat higher as the temperature is higher. For 10° and 15° its abscissa is 9.8 per cent., for 20° the corresponding oxygen pressure is shown as between 9.8 and 16 per cent., for 25° its abscissa is 20 per cent. and for 30° between 20 and 30 per cent.

The existence of two maxima with an intervening minimum in graphs of the sort shown in figure 6 and the first part of figure 7 seems not to have been pointed out in the literature before and the now obscure temperature-oxygen relations that must be responsible for these peculiar features are worthy of special study. An explanation is doubtless to be sought in the physiological chemistry of respiration in such seedlings as these, but no complete and plausible hypothesis seems as yet to be possible in this connection. An apparently pertinent suggestion will be presented after the graphs for shoot elongation have been considered. As has been mentioned, these two graph maxima imply a double optimal oxygen pressure for each temperature, the first (or lower) optimal pressure being in each instance the abscissa for the first graph maximum, while the second (or higher, main) optimal pressure is the abscissa for the second graph maximum. The physiological meaning of this apparently important double optimal pressure may be made clear by a concrete example, as follows: For the whole experiment period and the temperature of 25° the oxygen pressure of 9.8 per cent. gave a much higher rate of CO_2 production than was given either by 6.3 per cent. or by 20 per cent., while the rates for 6.3 and 20 per cent. are shown as about alike. The rate of CO_2 production for 25° and 6.3 per cent. of oxygen was 84.6 mg. and that for 25° and 20 per cent. of oxygen was 80.4 mg., while that for 25° and 9.8 per cent. of oxygen was 98.4 mg.

Graphs are shown here for only the second interval and the entire period, but the data of tables II and III show that the double graph maximum and the intervening minimum occur rather regularly for other intervals than the second, though the data are not wholly consistent in this respect. In table IV are assembled the approximate oxygen-pressure values corresponding to the first maximum, the second minimum and the main maximum in each graph, including the graphs not here reproduced as well as those of figure 6 and the first part of figure 7. The data are from tables II and III.

With two exceptions (30°, first interval, and 20°, second interval) the double graph maximum, which implies a double optimum in oxygen pressure, is at least suggested for all temperatures tested in all observation intervals and in the whole period and it is clearly shown for the higher temperatures in every case. Both the first optimal pressure (correspond-

TABLE IV

APPROXIMATE OXYGEN PRESSURES (PERCENTAGE OF OXYGEN IN THE GAS STREAM) CORRESPONDING TO FIRST OR SUBORDINATE MAXIMUM, SECOND OR SUBORDINATE MINIMUM AND SECOND OR MAIN MAXIMUM ON EACH GRAPH OF CO_2 PRODUCTION FOR EACH TEMPERATURE TESTED IN EACH OBSERVATION INTERVAL AND IN THE ENTIRE EXPERIMENT PERIOD. THE GRAPHS FOR THE SECOND INTERVAL AND THE ENTIRE PERIOD ARE REPRODUCED IN FIGURES 6 AND 7

		10°	15°	20°	25°	30°
		per cent.	per cent.	per cent.	per cent.	per cent.
1st interval	1st max.	16	6.3	9.8	9.8	
	2nd min.	20	9.8	16	20	
	2nd max.	75	90	90	90	90
2nd interval	1st max.	3.1?	6.3?		9.8	9.8
	2nd min.	6.3?	9.8?		20	30
	2nd max.	90	90	90	90	90
3rd interval	1st max.	6.3?	6.3	6.3	9.8	16
	2nd min.	9.8?	9.8	16	20	30
	2nd max.	75	95	90	90	90
4th interval	1st max.	6.3?	6.3	6.3	9.8	16
	2nd min.	9.8?	9.8	16	20	30
	2nd max.	90	95	95	95	95
5th interval	1st max.	6.3	6.3	9.8	16	16
	2nd min.	9.8	9.8	16	20	20
	2nd max.	90	95	95	95	95
Whole period	1st max.	3.1?	3.1	6.3	9.8	16
	2nd min.	9.8?	9.8	9.8—16	20	20—30
	2nd max.	90	95	90—95	95	90

ing to the first graph maximum) and the pressure corresponding to the second graph minimum are generally higher with higher temperature and with later intervals. Of course the pressure corresponding to the second minimum on a graph is always somewhat higher than that corresponding to the first maximum, the latter pressure being the one here called the first optimal pressure. Although some inconsistencies are to be noted, it seems clear from table IV that the double optimum of oxygen pressure for CO_2 production is significant and that the indications in this respect that are shown by the graphs of figures 6 and 7 are truly representative of the entire series of graphs.

The five graphs of CO_2 production in the whole period are also like those for the same process in the second interval in that each one has a

nearly rectilinear region lying between the second or subordinate minimum
and the second or main maximum; for oxygen pressures between about 20
or 30 per cent. and about 90 per cent., CO_2 production was nearly propor-
tional to oxygen pressure and the constant of proportionality (the upward
slope of the graphs) is progressively greater with higher temperature, its
value being apparently determined by a temperature and the current de-
velopment phase of the plantlets.

SHOOT ELONGATION IN THE ENTIRE EXPERIMENT PERIOD

Observations on the rates of enlargement exhibited by the seedlings of
the various cultures were not sufficiently precise for use in studying the
several observation intervals of the experiment period but the average final
length of the longest ten shoots of each culture furnishes a numerical value
that may represent the corresponding total shoot elongation for the entire
period. These average final lengths are given in table V. Where no value
is shown, elongation was too slight to be measured by the rather crude
method employed. All values in any line of the table are for the same
oxygen pressure and the highest value for each pressure is designated by
the use of bold-face type. An asterisk is shown at the left of the highest
growth value for each temperature (highest value in each column) and a
vertical line at the right brackets several values when these appear to
indicate an optimal range of oxygen pressure for the temperature in
question.

Besides the graphs of CO_2 production as related to temperature and
oxygen pressure in the entire experiment period, figure 7 presents also the
corresponding graphs for shoot elongation, the data for the latter being
taken from table V. Ordinates of these growth graphs are indices of total
shoot elongation for the entire experiment period and abscissae represent
percentages of oxygen in the gas mixture. There is a graph for each tem-
perature tested.

The graphs of shoot elongation differ from the corresponding ones of CO_2
production in several ways. While no main minimal oxygen pressure is
shown for CO_2 production with any temperature, all five of the graphs of
shoot elongation are seen to meet the axis of abscissae at the left. It is
important to note that the highest oxygen pressure giving no measurable
growth (a pressure slightly below the minimal pressure for shoot elonga-
tion) is progressively greater with higher temperatures. For 10° and 15°
this is 3.1 per cent., for 20° it is 6.3 per cent., for 25° it is 9.8 per cent.
and for 30° it is 16 per cent.

Each of these graphs of shoot elongation shows three low or minimal
regions and two high or maximal regions on the oxygen scale, as in the
case of the corresponding graphs of CO_2 production, but details are very

TABLE V

AVERAGE LENGTHS OF TEN LONGEST SHOOTS IN EACH CULTURE, AT END OF EXPERIMENT, AS RELATED TO TEMPERATURE AND PARTIAL PRESSURE OF OXYGEN

PARTIAL PRESSURE OF OXYGEN	AVERAGE FINAL LENGTH OF TEN LONGEST SHOOTS IN EACH CULTURE, FOR TEMPERATURE OF				
	10° C.	15° C.	20° C.	25° C.	30° C.
per cent.	mm.	mm.	mm.	mm.	mm.
0.6	..	... ..	......	....	......
3.1	... ... *	......	......	....	......
6.3	1.6	1.6	.....	. ...	......
9.8	2.4	2.8	2.6	....	......
16.0	2.8	3.8	3.0	2.4	......
20.0	3.0	4.8	7.2	7.2	2.2
30.0	3.0	5.0	*9.2	7.8	3.4
50.0	3.0	*5.4	9.0	11.0	3.8
75.0	2.4	4.0	6.6	8.0	4.4
90.0	2.2	4.6	8.4	10.6	4.0
95.0	2.6	4.8	8.4	*12.2	*9.2
98.3	2.0	3.6	6.8	10.0	6.8

* Highest growth value for each temperature.

different for the two processes. Each growth graph slopes sharply upward at the beginning, quickly attaining the first or subordinate graph maximum. Each graph then slopes downward to the second or subordinate graph minimum and then again upward to the second or main graph maximum, beyond which it slopes sharply downward to its end.

The presence of two graph maxima with an intervening subordinate minimum renders these graphs of shoot elongation peculiarly interesting and makes it necessary to consider (as in the case of the corresponding graphs of CO_2 production) two optimal oxygen pressures for each temperature. The remarkable similarity in form of all the growth graphs makes it seem probable that this feature is highly significant and important. (It is to be borne in mind, however, that the data of shoot elongation were secured by a much less precise technique than that by which the data of CO_2 production were secured.) These graphs all agree in showing the second or main maximum with an oxygen pressure of 95 per cent. (90–95 per cent. for

20°). The pressure of 98.3 per cent. was clearly supra-optimal for shoot elongation, as well as for CO_2 production, with every temperature. The first graph maximum for shoot elongation corresponds to a pressure range of 20–50 per cent. for 10°; to a pressure of 50 per cent. for 15° and 25°; to a pressure of 30 per cent. (or perhaps a pressure range of 30–50 per cent.) for 20°; and for 30° this maximum is suggested for a pressure of 75 per cent. The intervening second minimum is remarkably well and consistently shown by the graphs for 15°, 20° and 25°, on which it corresponds to a pressure of 75 per cent. It is indicated as corresponding to a pressure of 90 per cent. on the graphs for 10° and 30°, however, temperatures that are respectively below and above the temperature most favorable to growth. The 30-degree graph remains exceptionally low for all percentages of oxygen until the abscissa for 90 per cent. is passed, when it ascends sharply to its maximum, for 95 per cent.

As in the case of CO_2 production, no reference to a double optimum of oxygen pressure for growth seems to have appeared in the literature and this feature of growth control in seedlings such as were used in this study is worthy of special attention, notably in connection with the corresponding double optimum of oxygen pressure for CO_2 production and all that is implied by such occurrences.

SOME COMPARISONS BETWEEN CO_2 PRODUCTION AND SHOOT ELONGATION AS THESE WERE RELATED TO TEMPERATURE AND OXYGEN PRESSURE

POSSIBLE COMPETITION BETWEEN CO_2 PRODUCTION AND SHOOT ELONGA-TION.—In figure 7 it is seen that the pressure corresponding to the first or subordinate maximum on the graphs of CO_2 production for the whole experiment period is apparently the same as the highest oxygen pressure for which the corresponding growth graph has zero as ordinate. For the several temperatures these critical pressures are as follows: 10° and 15°, 3.1 per cent.; 20°, 6.3 per cent.; 25°, 9.8 per cent.; 30°, 16 per cent. CO_2 production was regularly more rapid with higher oxygen pressures up to the pressure that just allowed measurable shoot elongation, beyond which CO_2 production was notably lower with still higher pressures until the subordinate minimal region of the CO_2 graphs is passed. Although a considerable rate of CO_2 production may be supposed to be necessary for growth in such seedlings as these, yet the occurrence of growth may retard CO_2 production, as might be true if the two processes were competing, as it were, for the same plastic materials. The possibility that such competition may exist between growth and respiration were suggested by COPELAND (9) who said that "the more active the respiration, the less the relative amount of plastic material left available for growth." That the minimal oxygen pressure required for growth is greater with progres-

sively higher temperatures within the range considered, is in agreement with the results of CANNON's (7) experiments on the oxygen requirement of roots, and it appears that the higher temperatures may promote respiration more than they promote growth, other conditions being suitable. Of course the rate of CO_2 production should tend to increase with the growth rate when the latter is relatively great, for respiration must be supposed to proceed more rapidly as the number of active cells (or the volume of active protoplasm) increases with development and this may perhaps explain the second maxima in these curves. The highest percentage of oxygen tested generally gave somewhat lower rates of both shoot elongation and CO_2 production than did a slightly lower percentage with the same temperature. But neither growth nor CO_2 production approached zero rate with any tested temperature combined with the highest oxygen pressure tested, 98.3 per cent.

From the graphs in figure 7 it is apparent that the efficiency of respiration with respect to the amount of growth produced became greater as the oxygen pressure was increased above the main oxygen pressure minimum for growth, until the subordinate minimum of oxygen pressure for CO_2 production was passed. Above this region in the graph the efficiency of respiration gradually decreased until the subordinate minimum of oxygen pressure for growth was reached. For temperatures near the optimum for growth, the maximum efficiency of respiration was reached at oxygen pressures near that in ordinary air.

KOSTYTSCHEW points out (25, p. 54) that CO_2 production may not be greatly reduced by low partial pressures of oxygen in the surroundings, though the energy yield from respiration under such circumstances must be much less than when the oxygen pressure is higher. A rise in temperature may increase the respiration rate to a greater degree than it increases either the growth rate or the rate of transformation of plastic substances to available forms. The rate of growth may thus be limited on the one hand by the supply of available energy and on the other hand by the supply of plastic substances not used up in the respiration process.

TEMPERATURE INFLUENCE COMPARED WITH OXYGEN INFLUENCE WITH RESPECT TO CO_2 PRODUCTION AND SHOOT ELONGATION, BY MEANS OF COEFFICIENTS OF EFFECTIVENESS.—An idea of the relative influences of temperature and oxygen pressure on the rates of CO_2 production and shoot elongation, as shown by the results of these experiments, may be obtained by selecting an environmental complex that gave low rates and then ascertaining how much greater the rates were when one or the other or both of these two experimental variables were altered so as to give the greatest effect. What may be called coefficients of effectiveness (LIVINGSTON and SHREVE (29, p. 208) may be secured in this way for alterations of one or

more environmental conditions.   We may take as our unit for shoot elonga-
tion the value 3.0 mm., which corresponds, let us say, to the temperature-
oxygen combination of 10° and 20 per cent. (table V).   With the tem-
temperature increased from 10° to 25° (the oxygen concentration being
unchanged) the elongation rate was 7.2 mm., which is about 2.4 times our
unit.   On the other hand, when the temperature was not altered but the
oxygen pressure was increased from 20 per cent. to 95 per cent. the rate
value was only 2.6, which is lower than our unit, and the effectiveness of this
oxygen-pressure change is consequently negative, its coefficient being 0.9.
When both changes occurred together the rate was actually changed from
3.0 to 12.2 mm. and the coefficient of effectiveness of the double change is
4.7, which is 2.14 times as great as the calculated value, 2.2 (2.4 x 0.9).
If the two environmental changes applied together had operated just as
they did when applied separately, without mutual influence of antagonism
or synergism (*i.e.*, if the effects had been simply additive), then the rate
corresponding to the double change should have been 2.2 times as great
as our unit.   That the data actually show the coefficient of effectiveness
of the double change as 4.7 instead of 2.2 indicates that when both changes
were applied together the influence of one or both was greater than when
they were applied separately.   The two influences were synergistic in their
action and the synergism resulted in an increase of effectiveness from 2.2
to 4.7.

For CO_2 production (table III) these same changes give 3.5 and 1.5 as
the coefficients of effectiveness of the temperature alteration and of the
oxygen-pressure alteration, respectively, while the coefficient of the double
change is 8.3, which is 1.57 times the product of 3.5 and 1.5.   These con-
siderations show that the change from the temperature-oxygen combina-
tion of 10° and 20 per cent. to the combination of 25° and 95 per cent.
was relatively much more effective to give more rapid CO_2 production
than it was to give more rapid shoot elongation.   Also it is indicated that,
for both processes, the temperature change was much more important in
this double alteration than was the oxygen change and that there was
a pronounced synergistic action on both shoot elongation and CO_2 produc-
tion, this being nearly twice as great for CO_2 production as for shoot
elongation.

Such considerations as these may be valuable for comparisons when
further experimental data become available on the effectiveness of tem-
perature and oxygen pressure in controlling the rates of CO_2 production
and of growth in organisms.   For the present we may simply call attention
to the possibilities of this method of analysis and give emphasis to the con-
clusions just presented.

THE CARDINAL VALUES OF TEMPERATURE AND OXYGEN PRESSURE FOR
CO_2 PRODUCTION AND SHOOT ELONGATION IN THE ENTIRE
EXPERIMENT PERIOD

FOUR SETS OF VALUES CONSIDERED.—The relations of temperature and oxygen pressure to CO_2 production and shoot elongation may be studied by comparing the two sets of cardinal values for the two experimental variables. The values to be considered are (1) the main minimal, optimal and maximal temperature (A) for CO_2 production and (B) for shoot elongation, and (2) the same critical value of oxygen pressure. From the data of tables III and V (figure 7) the three cardinal values of temperature for each oxygen pressure and the three cardinal values of each oxygen pressure for each temperature are first to be ascertained as nearly as possible.

CARDINAL VALUES FOR CO_2 PRODUCTION.—The minimal temperature for CO_2 production in the entire period was evidently below 10° for every oxygen pressure tested, presumably for every oxygen pressure below that of pure oxygen under 1 atmosphere of gas pressure. The maximal temperature for CO_2 production in the whole period was evidently above 30° for all oxygen pressures, but 30° is surely somewhat supra-optimal for health in seedlings like these and it is probably safe to suppose that that temperature was about the maximum for CO_2 production *in health*. With the behavior of the seedlings under injurious or lethal conditions we are not here concerned.

The optimal temperature for CO_2 production in the whole period was 30° or above for all oxygen pressures tested, but because 30° is to be considered as generally supra-optimal for health we may say that the optimal temperature for CO_2 production *in healthy seedlings* was, like the corresponding maximal temperature, probably not far from 30° for all oxygen pressures below 100 per cent.

No main minimal oxygen pressure for CO_2 production in the whole period is shown for any temperature. It is clearly shown to have been at least below 0.6 per cent. in all cases, but it seems likely that there is no such thing as a minimal oxygen pressure for CO_2 production in such organisms as these, for CO_2 would probably be produced at a measureable rate in the complete absence of oxygen, at least with the higher temperatures tested.

No maximal oxygen pressure for CO_2 production in the whole period is shown and it is evident that we must consider this critical value as above 100 per cent. for all temperatures tested. It would of course be impossible to have an oxygen pressure above 100 per cent. without at the same time introducing a presumably serious alteration in the environmental background; namely, a total gas pressure of more than 1 atmosphere.

The highest rate of CO_2 production for each temperature is shown in black-face type in table III. Of course the oxygen pressure corresponding to the highest rate for any given temperature is the main optimal pressure for that temperature. This main optimum varied with temperature, being shown as 90 per cent. for 10° and 30° and as 95 per cent. for 15°, 20° and 25°. It is remarkable that this value is indicated as below that of pure oxygen for every temperature tested.

Special attention has already been given to the occurrence of a subordinate optimal and a subordinate minimal oxygen pressure for CO_2 production in the whole period, both with low values, which range from 6.3 and 9.8 per cent. (10° and 15°) to 16 and 20 per cent. (30°).

CARDINAL VALUES FOR SHOOT ELONGATION.—The minimal temperature for measurable shoot elongation in the entire period is seen (table V) to have been somewhat below 10° for every oxygen pressure for which data are available. This agrees with the corresponding observation for CO_2 production. But it is suggested that this minimal temperature for shoot elongation was probably lower for median pressures (20–50 per cent.) than for high or low pressures.

The maximal temperatures for measurable shoot elongation in the whole period is shown as low for low oxygen pressures and higher for higher oxygen pressures, being indicated between 15° and 20° for an oxygen pressure of 6.3 per cent., between 20° and 25° for a pressure of 9.8 per cent., between 25° and 30° for a pressure of 16 per cent., somewhat above 30° for pressure from 20 to 98.3 per cent. and farthest above 30° for a pressure of 95 per cent. These relations are very different from those for CO_2 production, for which no definite maximal temperature is shown for any oxygen pressure, although the temperature maximum was actually above 30° in every case.

The optimal temperature for measurable shoot elongation in the whole period is, like the corresponding maximal temperature, low for low oxygen percentages and higher for higher percentages. This optimum is indicated between 10° and 15° for a pressure of 6.3 per cent., about 15° for pressures of 9.8 and 16 per cent., about 20° for pressures of 20 and 30 per cent., and about 25° for pressures of 50–98.3 per cent. The temperature of 30° was unquestionably markedly supra-optimal for shoot elongation in the entire period, for every oxygen pressure employed. In this respect also the temperature relations of growths were very different from those of CO_2 production, for which no definite optimal temperature is shown for any oxygen pressure, although it was surely above 30° in every case.

The main minimal oxygen pressure for shoot elongation in the whole period varied with temperature, being higher for higher temperatures than for lower ones. It is indicated between 3.1 and 6.3 per cent. for 10° and

15°, between 6.3 and 9.8 per cent. for 20°, between 9.8 and 16 per cent. for 25°, and between 12 and 20 per cent. for 30°. This statement differs greatly from the corresponding statement concerning CO_2 production, for which no minimal oxygen pressure is shown for any temperature.

No maximal oxygen pressure for shoot elongation in the whole period is shown for any temperature and that maximum must have been above 98.3 per cent., if such a maximum existed. This is in agreement with the corresponding statement concerning CO_2 production.

The main oxygen-pressure optimum for shoot elongation in the whole period is shown at about 30 per cent. for 10°, probably between 30 and 50 per cent. for 15° and 20°, between 50 and 95 per cent. for 25° and about 95 per cent. for 30°. The oxygen pressure of 98.3 per cent. was clearly supra-optimal with respect to shoot elongation for every temperature tested. For CO_2 production this main optimal oxygen pressure is shown as 90 per cent. with 10° and 30°, and about 95 per cent. with temperatures from 15° to 25°.

The occurrence of two optimal oxygen pressures (or double optimum) for shoot elongation in the whole period has been considered above, where it is pointed out that CO_2 production and shoot elongation were apparently specifically related, in that the oxygen-pressure for shoot elongation is shown as having for each temperature the same value as the corresponding first optimal pressure for CO_2 production.

OPTIMAL COMBINATIONS OF TEMPERATURE AND OXYGEN PRESSURE FOR CO_2 PRODUCTION AND SHOOT ELONGATION.—The optimal temperature-oxygen combination for CO_2 production in the entire period, is shown as the combination of 30° and the pressure of 90 per cent., while the corresponding optimal combination for shoot elongation is shown as the combination of 25° and the pressure of 95 per cent. This constitutes an additional point of difference between the temperature-oxygen relations of the two processes. While the combination best suited to shoot elongation (25° and 95 per cent.) gave 12.2 mm. as the highest index of shoot elongation, the average mean hourly rate of CO_2 production corresponding to this index of elongation is only 190.8 mg., but the optimal combination for CO_2 production (30° and 90 per cent.) gave a corresponding CO_2 production of 232.6 mg. and the total amount of shoot elongation for that same combination of temperature and oxygen pressure is only 4.0 mm. per seedling for the experiment period. If the indices of shoot elongation (12.2 mm.) and CO_2 production (190.8 mg.) are both taken as unity for the combination of 25° and 95 per cent. the relative values of these indices for the combination of 30° and 90 per cent. become 0.3 (shoot elongation) and 1.2 (CO_2 production).

It is interesting to remark that the highest rate of shoot elongation shown may perhaps represent the developmental capacity of these seedlings,

for the background conditions and the duration factor employed. They had the capacity to develop shoots about 12.2 mm. long by the end of the experiment period if they were subjected to the temperature-oxygen combination of 25° and the oxygen pressure of 95 per cent. throughout the period, this development being accompanied by the production of about 1.9 mg. of CO_2 per seedling. None of the environmental complexes tested allowed any higher rate of shoot development but higher rates of CO_2 production actually occurred with combinations of 30° and an oxygen pressure of from 75 to 98.3 per cent. The capacity of these seedlings with respect to CO_2 production in the given period, and without regard to pathological or lethal phenomena probably related to the supra-optimal temperature of 30°, is shown as 232.6 mg. for 100 seedlings (or about 2.3 mg. per seedling), for the combination of 30° and the oxygen pressure of 90 per cent.

These observations constitute rather definite physiological characterizations of the seedlings used in this study and they may be valuable for purposes of comparison when systematic data of the sort presented in this paper shall have become available for other plant forms. We may suppose that any seed or seedling possesses a definite development or growth capacity, or a definite capacity for the production of CO_2, etc., and that an optimal environmental complex will permit the organism to attain its capacity, while failure to attain capacity in the specified time period implies a sub-optimal complex of environmental conditions. Of course it is logically possible that the optimal complex of conditions may be a broad range of complexes, or that there may be several different complexes each of which might allow or promote capacity performance. For performance considerably below capacity many different complexes may be suitable, as has been illustrated by the isopleths for CO_2 production in the second interval (figure 5), but as performance approaches the capacity of the organism the range of suitable complexes should become narrower. An interesting diagram of isopleths for shoot elongation may be constructed from the data of table V, but lack of sufficient space and the rather low degree of precision that characterizes these data have led to the omission of the growth diagram here.

It is possible that these seedlings might have exhibited a still greater capacity to grow or to produce CO_2 if the complex of environmental background conditions had been different; for example, if some toxic or stimulating gas were present in the gas stream. The capacity rate here emphasized is to be taken as holding, as far as present knowledge goes, only for the background complex used in these tests.

THE CARDINAL VALUES OF TEMPERATURE AND OXYGEN PRESSURE FOR CO_2 PRODUCTION IN THE SEVERAL OBSERVATION INTERVALS

In table II the highest rate of CO_2 production for each temperature is shown in bold-face type and of course these values correspond to the main optimal oxygen pressure for the respective temperatures. A study of these optimal pressures, in connection with the other cardinal values of oxygen pressure and the cardinal values of temperature, as they are alike or different for the several consecutive observation intervals, brings out a number of interesting relations, some of which will be mentioned below.

No minimal or maximal temperature with respect to CO_2 production is shown with any oxygen pressure tested (0.6 to 98.3 per cent.) in any of the five intervals. For all intervals, as for the whole period, the minimal temperature is shown as at least below 10°, and the maximal temperature is shown as above 30°, for every oxygen pressure tested. No optimal temperature is shown for any interval, but this value may be taken in every case as not far from 30° for all oxygen pressures, if only healthy seedlings are considered. Otherwise it may be considerably above 30°.

As in the case of the entire period, no main minimal oxygen pressure is shown for any interval or temperature; but this value was surely below 0.6 per cent. in every case. (As has been noted, there is reason for supposing that there is no such thing as a minimal oxygen pressure for CO_2 production in seedlings such as those here used.) No main maximum oxygen pressure is shown for any temperature in any interval, but this value was clearly above 98.3 per cent. in every case. The main optimal oxygen pressure is shown as varying with temperature and to some extent with the developmental phase of the plantlets. Its value is 90 per cent. for the first three intervals and 95 per cent. for the last two, for all temperatures tested, excepting that it is 75 per cent. for 10° in the second and 3rd intervals, 90 per cent. for 10° in the fourth and fifth intervals, 95 per cent. for 15° in the third interval.

In general, the later intervals are well represented by the second, for which the data have been presented rather fully by isopleths and plane graphs. The occurrence of a double optimum of oxygen pressure for CO_2 production is generally indicated for all temperatures in all intervals, the exceptions being of inconsistent occurrence or confined to temperatures that gave very low rates of CO_2 production with all oxygen pressures.

Summary and conclusion

NATURE OF THE EXPERIMENTATION.—This paper presents the results of an experimental study on CO_2 production and shoot elongation in very young wheat seedlings subjected to 60 different maintained environmental

complexes representing a wide range of temperatures and of partial pressures of oxygen in the culture solution in which the seedlings were submerged. The seedlings used were all very nearly alike, having been produced from selected seeds by means of a standard treatment. Their stage of development is indicated partly by the statement that the coleoptile was protruding less than a millimeter but that no roots had yet appeared, and partly by the specifications of the standard germination procedure that was regularly followed. One hundred seedlings were regularly used in each experiment.

The environmental background, including all the influential external conditions excepting the experimental variables, was very nearly the same for all experiments. This background portion of the maintained environment is described in detail, the main features being that the hundred seedlings of an experiment were submerged in 250 cc. of a standard, weak nutrient solution continually stirred by the passage of gas bubbles during the experiment period and that light was excluded from the culture chambers. The culture solution was not renewed and it doubtless changed in various ways (dependent on the activities of the seedlings) throughout the experiment period, which regularly extended for 46 hours after the close of a 2-hour period of transfer and adjustment, the previous germination period having lasted for 42 hours after the placing of the dry seeds in the water of the germination flask. The experiment period was subdivided into five consecutive observation intervals (of 4, 6, 12, 12 and 12 hours), for each of which the average hourly rate of CO_2 production was ascertained; there are thus available from each experiment five measurements of the rate of CO_2 production, representing different stages of the development of the seedlings, which of course changed internally as development advanced.

The experimental variables were maintained with very little fluctuation throughout the period of experiment, but they differed from experiment to experiment in a definite manner. They were the conditions of temperature and oxygen pressure, in 60 different combinations. Twelve different oxygen pressures were tested with each of five different maintained temperatures: 10°, 15°, 20°, 25°, 30° C. Any oxygen pressure was maintained by allowing a previously prepared mixture of oxygen and nitrogen to bubble continuously (at the rate of 20 cc. a minute) through the 250 cc. of nutrient solution in the culture flask. The twelve different oxygen-nitrogen mixtures contained 0.6, 3.1, 6.3, 9.8, 16, 20, 30, 50, 75, 90, 95 or 98.3 per cent. of oxygen by volume. Commercial gases were used in the preparation of the gas mixtures. The total gas pressure in the space above the solution in the culture flask was very little above the pressure of the outside atmosphere, the excess amounting to not more than 2 or 3 cm. of a mercury column.

Five experiments, all with the same partial pressure of oxygen but differing as to temperature, were carried out simultaneously in each experiment series and each series was repeated at least once before the final average rates of CO_2 production were computed. Some special repetitions near the end of the study showed clearly that the stock of seed used did not deteriorate appreciably while the work was in progress.

The final averages represent the rates of CO_2 production for the respective intervals, expressed as milligrams per hour, for 100 seedlings. The total amount of CO_2 produced by each culture was ascertained for each observation interval in each experiment period by absorbing the CO_2 in a measured quantity of standard $Ba(OH)_2$ solution, in an apparatus with some new features, and titrating the excess $Ba(OH)_2$ with standard HCl solution at the end of the interval. Observations on growth were made also and a numerical index of shoot elongation was secured from measurements made at the end of the entire experiment period.

PROGRESSIVE INCREASE IN CO_2 PRODUCTION WITH TIME.—The average rate of CO_2 production was generally greater for later observation intervals than for earlier ones in the same experiment. A number of exceptions to this general rule receive special attention. They are mainly confined to differences between rates for the first and second intervals and to combinations of temperature of 20° or below with oxygen percentages of 16 or below. Neglecting the exceptions, the increase in CO_2 production as time went on was generally slow for combinations of low temperature and low oxygen pressure, more rapid for combinations of low temperature and high oxygen pressure, and still more rapid for combinations of high temperature and high oxygen pressure. This acceleration in the rate of CO_2 production was most pronounced for the combination of 30° and an oxygen percentage of 90 or 95. To illustrate the increase in the rate of CO_2 production throughout the five intervals the following representative series of average hourly rates may be useful:

TEMPERATURE-OXYGEN COMBINATION	1ST INTERVAL	2ND INTERVAL	3RD INTERVAL	4TH INTERVAL	5TH INTERVAL
10°, 20 per cent........	0.39	0.41	0.45	0.53	0.61
20°, 20 per cent.........	0.82	0.84	1.07 ·	1.33	1.67
20°, 95 per cent...........	1.02	1.38	2.20	3.60	5.01
30°, 95 per cent...........	1.87	2.55	4.54	6.18	6.87

Special attention is given to the manner in which the rate of CO_2 production increased with the progress of the experiment when the seedlings were subjected to the temperature-oxygen combination of 20° and 20 per cent., a combination probably frequently encountered in nature. The average rates shown under these conditions for the five intervals are respectively: 0.82, 0.84, 1.07, 1.33 and 1.67 mg. and the acceleration is seen to be almost uniform. The rate is shown to have increased by about 0.025 mg. per hour during the 33-hour period between about the 9th hour and about the 42nd hour.

No MINIMAL OR MAXIMAL COMBINATION OF TEMPERATURE AND OXYGEN PRESSURE FOR CO_2 PRODUCTION IS SHOWN.—No culture failed to give measurable CO_2 production with any tested combination of temperature and oxygen pressure in any interval and consequently no minimal combination or maximal combination is shown for any interval or for the whole experiment period. The lowest rate for every interval is for the combination of 10° and an oxygen pressure of 0.6 per cent., which gave rates of 0.26, 0.27, 0.28 and 0.29 mg. per hour for 100 seedlings, for the second, third, fourth and fifth intervals, respectively. The combination of the highest temperature tested (30°) with the highest oxygen pressure tested (98.3 per cent.) gave high average rates in all intervals. These averages are 2.42, 4.07, 5.47 and 6.38 mg. per hour for 100 seedlings, for the second, third, fourth and fifth intervals, respectively.

OPTIMAL COMBINATION FOR CO_2 PRODUCTION.—The optimal combination of temperature and oxygen pressure for CO_2 production in the several intervals is shown as the combination of 30° and 90 per cent. (first three intervals) or 95 per cent. (last two intervals). In no case did the optimal combination include the highest oxygen pressure tested (98.3 per cent.). The highest rates shown for the second, third, fourth and fifth intervals are, respectively, 2.88 mg. (for 30° and 90 per cent.), 4.65 mg. (for 30° and 90 per cent.), 6.18 mg. (for 30° and 95 per cent.) and 6.87 mg. (for 30° and 95 per cent.).

RANGE OF POSSIBILITIES WELL REPRESENTED BY TEMPERATURES AND OXYGEN PRESSURES EMPLOYED.—The graphs and tables show that the 5-degree temperature intervals used in this study might have been narrower without the introduction of confusion due to unexplained variation among the different lots of seedlings, and some of the wider intervals of oxygen pressure might also have been reduced in width. On the whole, however, the intervals chosen for sampling the logical possibilities of combinations of temperature and oxygen pressure proved to be satisfactory for such a survey as this.

ISOPLETHS FOR EQUAL RATES OF CO_2 PRODUCTION IN THE SECOND INTERVAL.—The temperature-oxygen relations of CO_2 production in the second observation interval are set forth by a system of isopleths in a solid diagram and also by a set of plane graphs. The diagram shows clearly how the same rate of this process may result from many different combinations of temperature and oxygen pressure. For the region of this diagram between the lines for oxygen pressures of about 20 and about 90 per cent. the relation between temperature and oxygen pressure is shown to have been approximately linear for any rate value and the proportionality is about the same for all rates above 1.0 mg. Within this range the rate of CO_2 production for any oxygen pressure is shown to have been generally higher with higher temperature and the rate for any temperature was generally higher with higher oxygen pressure.

DOUBLE OPTIMAL OXYGEN PRESSURES FOR CO_2 PRODUCTION.—For the several intervals and also for the entire period two optimal oxygen pressures for CO_2 production are generally shown with each temperature, these being the abscissae for two distinct graph maxima. Between the two graph maxima there is of course a subordinate graph minimum. The first or subordinate optimal oxygen pressure and the pressure corresponding to the second or subordinate graph minimum are generally higher with higher temperatures; they generally vary from about 6.3 per cent. and 9.8 per cent. (for 10°) to about 16 per cent. and 20 per cent. (for 30°). The second or main optimal oxygen pressure is generally shown as 90 or 95 per cent. The physiological meaning of the double optimum of oxygen pressure for CO_2 production, a feature apparently not previously mentioned in the literature of plant respiration and one that seems to be important, may be illustrated by a concrete example from the data for 25° and for the whole experiment period, as follows: The oxygen pressure of 9.8 per cent. (first or subordinate optimum for 25°) gave a much higher rate of CO_2 production than was given either by 6.3 per cent. or by 20 per cent., while the rates for 6.3 and 20 per cent. were about alike. And a much higher rate was given by 95 per cent. (main optimum for 25°) than by 9.8 per cent. The totals for these critical pressures, for 25° and for the entire period, are shown below.

Lowest oxygen pressure tested (0.6 per cent.) .... 46.0 mg.
First or subordinate optimal pressure (9.8 per cent.) ...... 98.4 mg.
Second or subordinate graph minimum (20 per cent.) 80.4 mg.
Second or main optimal pressure (95 per cent.) .... 190.8 mg.

Neither for any of the observation intervals nor for the whole period did the highest oxygen pressure tested (98.3 per cent.) give the highest rate

of CO_2 production for any temperature, and the pressure 98.3 per cent. was consequently supra-optimal for CO_2 production in every instance.

PROPORTIONALITY OF CO_2 PRODUCTION TO OXYGEN PRESSURE.—As in the case of the second and later intervals, CO_2 production in the whole period is shown for any temperature as about proportional to oxygen pressure within the pressure range from that for the subordinate graph minimum to that for the main maximum; *i.e.*, between about 20 or 30 per cent., and about 90 or 95 per cent. The constant of proportionality is shown to be greater with higher temperature.

CARDINAL TEMPERATURES FOR SHOOT ELONGATION.—The data for shoot elongation in the entire experiment period are discussed in detail and their relations are compared to the corresponding relations of CO_2 production. No main minimal temperature is indicated for shoot elongation; it was clearly below 10° for every oxygen pressure tested. On the other hand, a minimal oxygen pressure for measurable shoot elongation in the experiment period is shown for every temperature tested and this critical pressure was progressively greater with higher temperature. The lowest pressures giving measurable shoot elongation were: for 10° and 15°, 6.3 per cent.; for 20°, 9.8 per cent.; for 25°, 15 per cent.; for 30°, 20 per cent.

DOUBLE OPTIMAL OXYGEN PRESSURES FOR SHOOT ELONGATION.—The graph for shoot elongation with each temperature generally shows three low or minimal regions and two high or maximal regions, when oxygen pressures are abscissae and indices of shoot elongation are ordinates. It is therefore necessary to consider a double optimum of oxygen pressure for each temperature, as in the case of CO_2 production, but the details are very different for the two processes. Such a double optimum of oxygen pressure seems not to have been recorded in the literature of plant growth and it appears to be important.

The first graph maximum for shoot elongation corresponds to a pressure range (first or lower optimal pressure) of 20–50 per cent. for 10°; to a pressure of 50 per cent. for 15° and 25°, to a pressure range of 30–50 per cent. for 20° and to a pressure of perhaps 75 per cent. for 30°. The second or main graph maximum corresponds to a pressure (second, upper, or main optimal pressure) of 90 or 95 per cent. for every temperature. The intervening second or subordinate graph minimum corresponds to a pressure of 75 per cent. with temperature of 15°, 20° and 25° and to a pressure of 90 per cent. with 10° and 30°.

It is remarkable that, for every temperature tested, the first or main minimal oxygen pressure for shoot elongation is shown as coincident with

the corresponding first optimal pressure for CO_2 production. CO_2 production in the whole period was regularly more rapid with higher oxygen pressure up to the pressure that just allowed measurable shoot elongation, beyond which CO_2 production was notably lower with still higher oxygen pressures until the subordinate minimal region of the CO_2 graph is passed. Although a considerable rate of CO_2 production may be supposed to have been necessary for growth, yet the occurrence of growth may have retarded CO_2 production, as might be true if the two processes were competing for the same plastic materials.

The highest oxygen pressure tested (98.3 per cent.) was supra-optimal for shoot elongation, as well as for CO_2 production, with every temperature employed.

EFFECTIVENESS OF TEMPERATURE AND OXYGEN PRESSURE IN DETERMINING CO_2 PRODUCTION AND SHOOT ELONGATION.—The relative magnitudes of the temperature influence and the oxygen influence on the rate of CO_2 production and shoot elongation in the whole experiment period are examined by means of coefficients of effectiveness for environmental differences. Within the range of temperature-oxygen combinations studied, the temperature influence was much greater than the oxygen influence with respect to both processes, and both influences were more pronounced with respect to CO_2 production than with respect to shoot elongation. The two influences were generally synergistic when operating together; i.e., the effect of a temperature difference and a simultaneous oxygen-pressure difference was greater than the summation of the effects of the two differences considered separately.

CARDINAL TEMPERATURES FOR CO_2 PRODUCTION AND SHOOT ELONGATION COMPARED.—The main cardinal values (minimum, maximum and optimum of temperature and of oxygen pressure) for CO_2 production and for shoot elongation in the whole experiment period, are studied and the two processes are compared with respect to these values. The minimal temperature for both processes was below 10° for every oxygen pressure for which data are available. The maximal temperature for CO_2 production in the whole period is shown as above 30° for every oxygen pressure tested, while for measurable shoot elongation it varied directly with the oxygen pressure; this maximum is shown as between 15° and 20° for an oxygen pressure of 6.3 per cent., between 20° and 25° for a pressure of 16 per cent., somewhat above 30° for pressures of 20–98.3 per cent. and farthest above 30° for a pressure of 95 per cent. The optimal temperature for CO_2 production was actually above 30° for every oxygen pressure tested but for shoot elongation this optimum varied directly with the oxygen pressure in the lower portion

of the pressure range. It is shown as 10°–15° for a pressure of 6.3 per cent., 15° for pressures of 9.8 and 16 per cent., 20° for pressures of 20 and 30 per cent. and 25° for pressures of 50–98.3 per cent.

MAIN CARDINAL OXYGEN PRESSURES FOR CO_2 PRODUCTION AND SHOOT ELONGATION COMPARED.—There is probably no such thing as a main minimal oxygen pressure for CO_2 production with any temperature, but for measurable shoot elongation in the experiment period the main minimal oxygen pressure is shown to have varied with temperature, being higher with higher temperature, as has been mentioned. The main maximal oxygen pressure for each temperature is shown as above 98.3 for both processes and attention is called to the consideration that an oxygen pressure higher than 100 per cent. would be impossible of attainment unless the total gas pressure were above 1 atmosphere. With respect to the main optimal oxygen pressures, as has been noted, the two processes differed very much. For CO_2 production this main oxygen pressure optimum was 90 per cent. with 10° and 30° and 95 per cent. with 15°, 20° and 25°, but it varied with temperature in quite another manner for shoot elongation.

The optimal combination of temperature and oxygen pressure for CO_2 production in the whole period is shown as the combination of 30° and 90 per cent. while the corresponding optimal combination for shoot elongation is shown as the combination of 25° and 95 per cent.

It is remarked that the highest rates of CO_2 production for the entire period and the corresponding total shoot elongation probably approximate the capacity of these seedlings for the given period. Thus, they had the capacity to develop shoots 12.2 mm. long by the end of the period if subjected to the optimal combination of temperature and oxygen pressure for this process, and they had the capacity to produce 232.6 mg. for 100 seedlings by the end of the period if subjected to the optimal combination for CO_2 production. These are physiological characterizations of the seedlings used in this study.

CARDINAL TEMPERATURES AND OXYGEN PRESSURES FOR CO_2 PRODUCTION IN THE SEVERAL INTERVALS.—With regard to the cardinal values of temperature and oxygen pressure for CO_2 production in the several observation intervals, no minimal or maximal temperature is shown for any oxygen pressure in any interval. For all intervals, as well as for the whole period, these critical values are shown as respectively below 10° and above 30° for every oxygen pressure tested. As in the entire experiment period, no optimal temperature is shown for any oxygen pressure in any interval, and this value is indicated as above 30° in all instances, but that temperature was surely supra-optimal for health. No main minimal oxygen pressure is shown for

any temperature in any interval but this value was surely below 0.6 per cent. in every case, which is also true of the whole period.   As has been noted, it is probable that there is no such thing as a main minimal oxygen pressure for CO_2 production with any temperature for seedlings such as these in any phase of their development.   No main oxygen-pressure maximum for CO_2 production is shown for any temperature in any interval, but this was clearly above 98.3 per cent. in every case, just as for the entire period.   On the other hand, the main optimal oxygen pressure for CO_2 production varied not only with temperature (as shown for the whole period) but also to some extent with the developmental phase of the plantlets.   This main pressure optimum is generally shown as 90 per cent. for the first three intervals, and 95 per cent. for the last two intervals for all temperatures tested.

A FUNDAMENTAL PRINCIPLE EMPHASIZED.—The results of this whole study illustrate the most fundamental consideration for all research on the natural determination of process rates; namely, that the relation of any process to any influential condition or influence cannot be definitely stated without quantitative reference to the other prevailing influences.   This is of course because of the inevitable concomitancy and interaction of all the influential conditions of the current environmental complex, including the time or duration factor.   This general principle, which is basic for all physiological and ecological analysis, has been emphasized by BLACKMAN (4) in the following words: "The way of those who set out to evaluate exactly the effects of changes in a single factor upon a multi-conditioned metabolic process is hard, and especially so when this process is being pushed towards the upper limits of its activity."

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LITERATURE CITED

1. AMBERSON, W. R., MAYERSON, H. S., and SCOTT, W. J.   The influence of oxygen tension upon metabolic rate in invertebrates.   Jour. Gen. Physiol. 7: 171–176.   1924.

2. BERGMAN, H. F.   The respiratory activity of various parts of the cranberry plant in relation to flooding injury.   Amer. Jour. Bot. 12: 641–659.   1925.

3. BERT, P.   La pression barométrique.   Chapt. 5.   Influence des modifications de la pression barométrique sur les végétaux.   p. 845–866.   1878.

4. BLACKMAN, F. F.   Optima and limiting factors.   Ann. Bot. 19: 281–295.   1905.

5. BLANC, L. Recherches expérimentales sur l'influence des variations de température sur la respiration des plantes. Rev. Gen. Bot. **28**: 65–79. 1916.

6. BÖHM, Jos. Über das Keimen von Samen in reinem Sauerstoffgase. Sitzungsber. d. math.-naturw. Klasse Wiener Akad. Wiss. **68**: 132–141. 1873.

7. CANNON, W. A. The influence of the temperature of the soil on the relation of roots to oxygen. Science n.s. **58**: 331–332. 1923.

8. CARRICK, D. B. The effect of freezing on the respiration of the apple. Cornell Univ. Agr. Exp. Sta. Memoir 110. 1–28. 1928.

9. COPELAND, E. B. Chemical stimulation and the evolution of carbon dioxide. Bot. Gaz. **35**: 81–98 and 160–183. 1903.

10. DÖBEREINER, J. W. Expériences sur la germination dans l'air condensé ou rarefié. Biblioth. Univ. de Geneve **22**: 121. 1823.

11. FAWCETT, H. S. The temperature relations of growth in certain parasitic fungi. Univ. California Pub. Agr. Sci. **4**: 183–232. 1921.

12. GODLEWSKI, EMIL. Beiträge zur Kenntniss der Pflanzenathmung. Jahrb. wiss. Bot. **13**: 491–543. 1882.

13. HAASIS, F. W. Germinative energy of lots of coniferous-tree seed, as related to incubation temperature and to duration of incubation. Plant Physiol. **3**: 365–412. 1928.

14. HARRINGTON, G. T. Forcing the germination of freshly harvested wheat and other cereals. Jour. Agr. Res. **23**: 79–100. 1923.

15. HÉE, A., and BONNET, R. Influence de la teneur en oxygène de l'eau sur la respiration des plantes submergées. Compt. Rend. Acad. Sci. Paris **180**: 457–459. 1925.

16. HEUMANN, M. Growth acceleration in plants under decreased oxygen pressures [translated title]. Bot. Arch. **4**: 413–443. 1923.

17. VON HUMBOLDT, F. ALEXANDER. Aphorismen aus der chemischen Physiologie der Pflanzen. Übers. von GOTTHELF FISCHER. Leipzig, 1794. p. 68–69.

18. HUTCHINS, L. M. Studies of the oxygen-supplying power of the soil together with quantitative observations on the oxygen supplying power requisite for seed germination. Plant Physiol. **1**: 95–150. 1926.

19. JOHANNSEN, W. Über den Einfluss hoher Sauerstoffspannung auf die Kohlensäureausscheidung einiger Keimpflanzen. Untersuchungen aus dem bot. Inst. Tübingen **1**: 686–717. 1885.

20. KANITZ, ARISTIDES. Temperatur und Lebensvorgänge. Berlin. 1915.

21. KIDD, F. The controlling influence of carbon dioxide in the maturation, dormancy, and germination of seeds. Part I. Proc. Roy.

Soc. London B **87**: 408–421. 1914. Part II. Proc. Roy. Soc. London B **87**: 609–625. 1914. Part III. The retarding effect of carbon dioxide on respiration. Proc. Roy. Soc. London B **89**: 136–156. 1915.

22. —————————. Laboratory experiments on the sprouting of potatoes in various gas mixtures. New Phytol. **18**: 248–252. 1919.

23. —————————, and WEST, C. A relation between the concentration of oxygen and carbon dioxide in the atmosphere, rate of respiration, and length of storage life in apples. (Gt. Brit.) Dept. Sci. and Indus. Res., Food Invest. Bd. Rept. 1925–1926. pp. 41–42. 1925–1926.

24. —————————. Retardation of the ripening of pears by the exclusion of oxygen. Nature **123**: 315–316. 1929.

25. KOSTYTSCHEW, S. Pflanzenatmung. Berlin. 1924. (See also KOSTY-CHEV's Plant Respiration, ed. by C. J. LYON.)

26. LEHENBAUER, P. A. Growth of maize seedlings in relation to temperature. Physiol. Res. **1**: 247–288. 1914.

27. LIVINGSTON, B. E. A plan for cooperative research on the salt requirements of representative agricultural plants. Prepared for a special committee of the Division of Biology and Agriculture of the National Research Council. 2nd ed. edited by Burton E. Livingston, Baltimore, 1919.

28. —————————, and FAWCETT, H. S. A battery of chambers with different automatically maintained temperatures. Phytopath. **10**: 336–340. 1920.

29. —————————, and SHREVE, FORREST. The distribution of vegetation in the United States, as related to climatic conditions. Carnegie Inst. Washington Publ. no. 284. 1921.

30. LOVE, H. H. The importance of the probable error concept in the interpretation of experimental results. Jour. Amer. Soc. Agron. **15**: 217–224. 1923.

31. NOMURA, SHICHIROKU. The influence of oxygen tension on the rate of oxygen consumption in *Caudina*. Sci. Rept. Tohoku Imp. Univ. **2**: 133–138. 1926.

32. PALLADIN, W. I. Plant Physiology. Third Amer. ed., edited by B. E. LIVINGSTON. Philadelphia, 1926.

33. —————————. Influence des changements de température sur la respiration des plantes. Rev. Gen. Bot. **11**: 241–257. 1899.

34. PARIJA, P. Analytical studies in plant respiration II. The respiration of apples in nitrogen and its relation to respiration in air. Proc. Roy. Soc. London B **103**: 446–490. 1928.

35. Pfeffer, W. Physiology of plants. 2nd ed., edited by A. J. Ewart.
    Vol. 2: 114–115. Oxford, 1903.

36. Rollo, John. Expériences et observations sur le sucre. Ann. de chim.
    25: 37–50. 1798.

37. de Saussure, Th. Recherches chimiques sur la végétation (1804).
    Chemische Untersuchungen über die Vegetation. Ostwald's Klas-
    siker der Exakten Wissenschaften, nos. 15: 2, pp. 9–13; 16: pp.
    20–22. Leipzig, 1890.

38. Scheele, C. W. Chemische Abhandlungen von Luft und Feuer (1777)
    W. Ostwald's Klassiker der exalten Wissenchaften no. 58, p. 87.
    Leipzig, 1894.

39. Shive, J. W. A study of physiological balance in nutrient media.
    Physiol. Res. **1**: 327–397. 1915.

40. ——————, and Stahl, A. L. Constant rates of continuous solution
    renewal for plants in water cultures. Bot. Gaz. **84**: 317–323.
    1927.

41. Shoup, Charles S. The respiration of luminous bacteria and the
    effect of oxygen tension upon oxygen consumption. Jour. Gen.
    Physiol. **13**: 27–45. 1929.

42. Spoehr, H. A., and McGee, J. M. The effect of fluctuations in the CO_2
    content of the atmosphere on the rate of respiration of leaves.
    Amer. Jour. Bot. **11**: 493–501. 1924.

43. Stich, Conrad. Die Athmung der Pflanzen bei verminderter Sauer-
    stoffspannung und bei Verletzungen. Flora. **74**: 1–57. 1891.

44. Treadwell, F. P., and Hall, W. T. Analytical chemistry. Vol. II.
    Quantitative. 1924.

45. Trelease, S. F., and Livingston, B. E. Continuous renewal of nu-
    trient solution for plants in water cultures. Science n.s. **55**: 483–
    486. 1922.

46. Wilson, W. P., and Pfeffer, W. Über intramolekulare Athmung.
    Untersuchungen aus dem bot. Inst. Tübingen. **1**: 638–685. 1885
    (p. 655).

47. Wurmser, R., and Jacquot, R. Sur la relation entre l'état physique
    du protoplasma et son fonctionnement. I. Photosynthèse. Bull.
    Soc. Chim. Biol. **5**: 305–315. 1923.

RELATION OF INCREASED WATER CONTENT AND DECREASED AERATION TO ROOT DEVELOPMENT IN HYDROPHYTES

J. E. WEAVER AND W. J. HIMMEL

(WITH ELEVEN FIGURES)

The dependence of root development of most plants upon aeration is clearly shown by water-logging the soil. In a few days, the usual cultivated plants turn yellow, show wilting, and may ultimately die. If the water is kept well aerated, plants may survive though submerged for weeks. Exclusion of oxygen from the roots of most plants interferes with the respiration of the protoplasm of their cells. This results in the death of the cells and consequently the roots fail to function as absorbers for the plant (22).

The importance of aeration in plant development has long been recognized by plant physiologists and a very large number of investigations have been made. In 1921, CLEMENTS (7) summarized nearly 700 such papers in his mongraph on "Aeration and Air-Content." The long-continued work of CANNON has added much to an understanding of this subject and his "Physiological Features of Roots" (5) includes the most extensive recent contribution to this problem. The importance of aeration in the production of crops and the growth of forests has been emphasized by the extensive researches of HOWARD (15), HOWARD and HOWARD (16, 17), and HOLE (14) in India. A survey of the literature shows that, with rare exceptions, there is general agreement that the aeration of the roots promotes a better growth of tops. Most of the experiments were carried on in water cultures, e.g., HALL, BRENCHLEY and UNDERWOOD (12), ANDREWS and BEALS (1), and BERGMAN (3), and some in soil, such as those of HUNTER (19), HOLE (14), HOWARD (15), and KNIGHT (21). The response of the plant in most cases has been measured by the development of tops and little account has been taken, especially in soil cultures, of the organs directly affected by the increased aeration. The work of BALLS (2), HUNTER (19), HOLE (14), and the HOWARDS (16, 17) are among outstanding exceptions. CANNON (5) measured the effect of inadequate aeration on root elongation and not on the development of the root system as a whole.

In devising a simple experiment for classes in plant physiology and ecology to illustrate the effect of aeration upon root and root-hair development, *Typha latifolia* was employed (24). It was selected because it grows naturally under a wide range of conditions, from water-logged soil to one that has a sufficiently low water content to be well aerated. Differences in root response under conditions of poor and good aeration were so marked

that three other characteristic and widely distributed swamp and swamp-margin plants were also employed and an extensive study of their root behavior begun. The use of large containers furnished an opportunity to determine the normal nature and extent of the underground parts, a procedure which met with almost insurmountable difficulties where the plants grow naturally in soil covered with water.

Procedure

Rhizomes of *Typha latifolia, Scirpus validus,* and *Spartina michauxiana* were dug in quantity on April 5, before new growth had begun. They were kept in a refrigerator for a period of 14 days before planting on April 19. *Phragmites communis* was taken from a swamp at this time and immediately transplanted. Growth was just starting.

Four large galvanized iron containers, 30 cm. square in cross section, were used for each species. The following conditions of soil moisture and concomitant aeration were maintained: (1) saturated, no free air; (2) alternately saturated and drained, aeration poor; (3) optimum water content for land plants, aeration good; and (4) dry soil, aeration good. The depth of the containers necessary to accommodate maximum root growth was determined by preliminary experiments; those for conditions 1 and 4 were 60 cm. deep, the others 90 cm. A well screened, fertile, loam, potting soil was used. It was mixed with one-third of its volume of sand. This mixture had a hygroscopic coefficient of 10 per cent. and a water holding capacity (HILGARD method) of about 55 per cent.

In filling the saturated containers, the soil was thoroughly saturated and constantly stirred to permit the escape of air. After the soil had settled, an excess of water was added to the container so that a layer of clear water 2.5 to 5 cm. deep stood constantly above the soil. Losses by evaporation were replaced every two or three days by adding tap water from a sprinkler. Six liters of water were daily poured on the gravel mulch of the drained soil. The water slowly sank through the soil mass and disappeared from the surface after 10 to 30 minutes. It slowly trickled through 2.5 cm. of coarse gravel placed on the bottom and out through an opening on the side. Here it was caught in a container and again used. This prevented loss of nutrients or other substances from the soil by leaching. Since there was some loss by evaporation, fresh tap water was added from time to time as needed. The third set of containers had soil of optimum water content (25 per cent. based on dry weight) which was lightly tamped in filling. The soil was covered with a fine gravel mulch, as was the case with all the containers except the first lot. Only enough water was added to replace that actually lost by transpiration and surface evaporation. The dry con-

tainers were three-fourths filled with a dry soil which had only 12 per cent. water content. This was but 2 per cent. in excess of the hygroscopic coefficient. Above this was placed a 15-cm. layer of soil with a water content of 25 per cent. Only enough water was added to the upper soil to insure continued growth.

Before planting, the rhizomes were carefully selected and cut in such a manner that they were quite uniform in size, number of sprouts, etc. All of the roots were cut close to the rhizome and seven rhizomes were planted at depths of 3.75 to 7.5 cm. (varying with the species) in each container.

FIG. 1. Development of plants 17 days after transplanting the rhizomes. The sequence from left to right is dry, saturated, moist, and drained soil.

All of the plants, except *Phragmites*, rapidly developed. The growth of the reed, although transplanted when still young, was somewhat checked compared with the development of plants in the swamp (fig. 1). For the sake of clarity each species will be separately considered.

Results

TYPHA LATIFOLIA

The development of the aboveground parts of *Typha*, at the end of the 35 days they were permitted to grow, is shown in table I.

TABLE I

GROWTH OF *Typha latifolia* ABOVE GROUND WITH DIFFERENT MOISTURE CONDITIONS

CONDITION FOR GROWTH→	SATURATED SOIL	DRAINED SOIL	MOIST SOIL	DRY SOIL
Maximum height, cm.	111.0	106.0	86.0	66.0
Average number of leaves	8.4	8.1	7.6	7.0
Average height of leaves, cm.	46.8	42.3	33.0	27.5
Average width of leaves, mm.	13.0	12.4	9.5	9.4
Total dry weight of tops, grams	16.2	19.9	12.2	7.2

An examination of the table shows that in every way development decreased with a decrease in water content. The dry weight is only an apparent exception, since in the wet culture only 5 plants grew, but there were 7 in each of the others, except the dry soil where one plant died. Here, as among the other three species, the plants in the moist and dry soils had a darker green color throughout their period of growth, due probably, to the more favorable conditions for nitrification and nitrogen fixation. In the saturated and drained containers the roots were readily freed from adhering soil particles and their dry weight was obtained. The dry weight of tops and roots was in the ratio of 5.8 to 1 in the saturated soil and 5.6 to 1 in the drained.

SATURATED CULTURE.—The root systems were examined on May 23–25 when the plants were five weeks old. Those in the saturated soil were very shallow and consisted of two distinct parts, namely, soil roots and water roots. All originated from a basal node of the stem about 6 cm. below the soil surface and none from the old rhizome. The rhizomes shriveled upon the removal of accumulated food and in several cases they were decomposing, although the soil was not sour. The soil roots, 64 in number on one of the largest plants, pursued courses at all angles between the horizontal and the perpendicular in such a manner as to more or less thoroughly ramify a hemisphere of soil with a radius of 15 cm. Eleven of the longest extended to 28 cm. depth.

These shining white roots were 1.5 to 2 mm. in diameter and very turgid and brittle, especially the distal 10 cm. Branches were entirely absent on most of the roots or occurred only very rarely. On others, slightly brownish in color and probably older, thread-like laterals arose at the rate of 1 to 8 per cm. (rarely 14 on the best branched parts). These were exceedingly variable in their distribution, frequently long portions of the roots being bare. Laterals were 1 to 5 cm. (maximum 10 cm.) long, and entirely unbranched but much more abundantly furnished with root hairs than the main roots where they were relatively sparse.
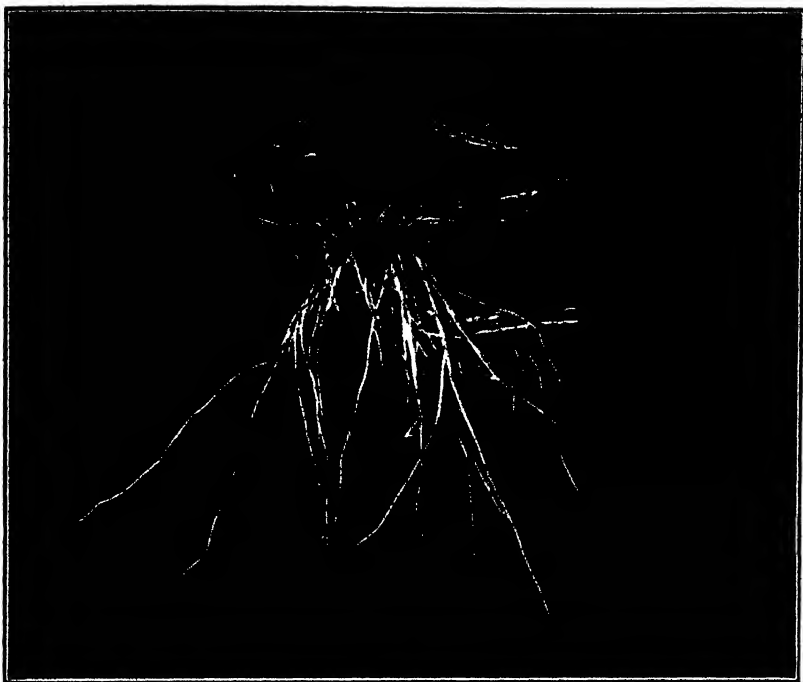
FIG. 2. *Typha latifolia* grown in soil covered with standing water, showing the sharp differentiation between soil and water roots. Photograph taken with root system under water in its natural position.

Approximately half of the root system (66 roots by actual count) consisted of water roots. These arose at a higher position on the node than most of the soil roots. While some ran obliquely upward, often at a 45° angle, many grew vertically and then turned at right angles after leaving the soil (fig. 2). Other roots ran horizontally about 8 cm. and then grew abruptly upward. The portions in the soil were like those described, but upon entering the water they greatly decreased in diameter and branched profusely, the distal part becoming extremely attenuated (figs. 3 and 4). They began to appear only 5 days after the rhizomes were planted and their growth was rapid, some reaching a length of 10 cm. in 3 days. By the expiration of 10–14 days all were greatly branched with laterals a centimeter or less in length. New roots continually appeared and branching increased throughout the period of growth. The older ones reached a maximum length of 30 cm. but many of them were only about one-half as long. When mature, they were branched to near the tips but there were no laterals of the second order. The very fine primary laterals, which occurred at the rate of 20 to 55 per cm., varied in length from 1.5 to 2.5 cm. and were en-

FIG. 3.   Water roots of *Typha* about two weeks after transplanting the rhizomes.

tirely free from root hairs.   These fine water roots and their very abundant branches enormously increased the absorbing surface in the aerated portion of the culture.   Aside from frequent renewal of the water, an abundance of *Ulothrix* and *Mougeotia* increased the oxygen content.   The former alga was so abundantly attached to the roots that it gave them a greenish hue.

DRAINED CULTURE.—The root system in the drained soil was much deeper seated although the roots were most abundant and most branched in the surface 9 cm., which was the best aerated.   Here they formed a dense

1 cm

FIG. 4.   Detail of water root of *Typha*.   Note the attenuated tip and the profuse simple branching.

network.   Roots were numerous to a depth of 30 cm. and some attained depths of 40 to 47 cm. (fig. 5).   The general distribution of the roots was such as to occupy a more or less hemispherical mass of soil.   They were rather clearly differentiated into three types as regards position, thickness, and degree of branching.
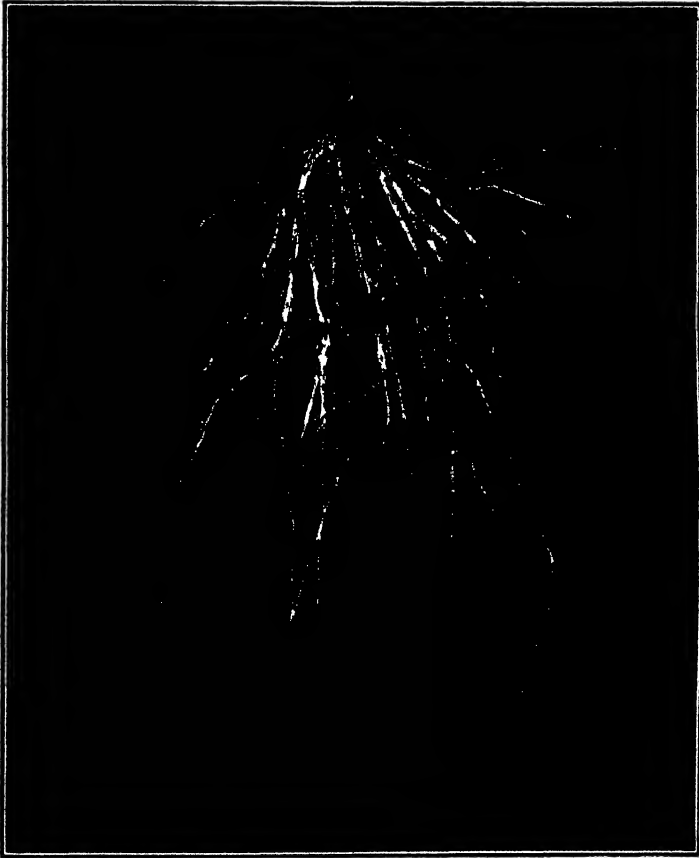
Fig. 5.   *Typha* grown in drained soil.   The finer, horizontal roots are near the soil surface.

One lot consisted of coarse, unbranched or practically unbranched roots which, as a group, penetrated deepest.   Their diameters were 1.5 to 2 mm. throughout.   A second lot was composed of roots with smaller diameters (1–1.5 mm.), some of which penetrated as deeply, or at least nearly as deeply, as the former.   These branched profusely, often forming networks of laterals at depths of 30–36 cm. where branches 10 cm. long were found. The branching habit was variable; on some, only the proximal portion was

branched, on others, the distal part. On still others the presence of branches and especially their density varied from place to place. The rate of branching was 6 to 25 laterals per cm. The branches ranged in length from less than 1 cm. to a maximum of 10 cm., although most of them did not exceed 3 cm. Nearly all of the primary laterals were simple; a few were branched to the first order, the branches seldom exceeding 3 or 4 per lateral, and a centimeter in length.
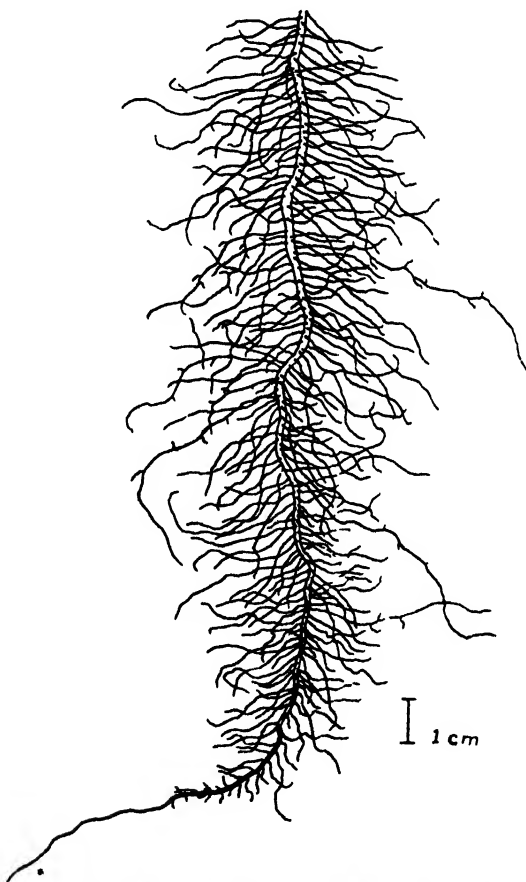
FIG. 6. Detail of one of the surface roots of *Typha* grown in drained soil.

A third type of root arose from the highest portion of the node at the base of the stalks and thus above the other roots. They thus originated at a depth of about 7 cm. and no portion of the root extended deeper than the point of origin. In fact, a few at first extended vertically upward but this tendency was not nearly so marked as in the case of water roots in the previous culture. Branches of these fine roots (which were 0.5 to 1 mm. thick) often occurred throughout, but in general they were most abundant and

longest on the distal one-third to one-half. Laterals were 7 to 10 per cm. on the more thinly branched parts and 12 to 20 on those most thickly branched. These thread-like roots were shining white, and the longest were more abundantly furnished with laterals than were any in the deeper soil. Secondary branches were few. The main root and all its branches were well supplied with root hairs. These roots differed from the water type by having longer, coarser, and more crinkly laterals with some sublaterals and an abundance of root hairs (fig. 6). The last was in striking contrast to the water roots, which had none.

One of the larger plants had 56 of the deep, unbranched type, 33 of the deeply penetrating branched ones, and 43 of the fine, surface type which were most favorably situated as regards aeration.

MOIST CULTURE.—The root system in the moist, well aerated soil showed no differentiation into parts; all of the roots were 1 to 1.5 mm. in diameter and about equally branched. There were no roots in the surface 7 cm. of soil. Long horizontal ones ran outward, sometimes to distances of 30 cm., but never turned downward. Others ran outward and downward to depths
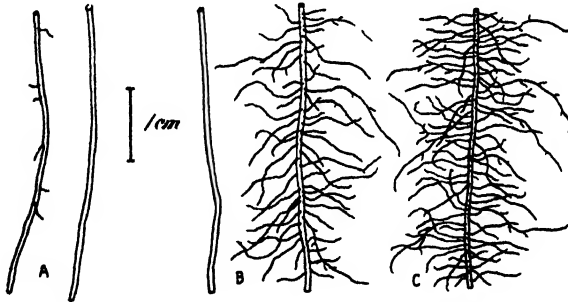
FIG. 7. Relative branching of roots of *Typha*; A, in saturated soil; B, in wet but drained soil; and C, in moist, well aerated soil.

of 40 to 53 cm. All, except the shortest, were regularly branched except the rapidly growing, shining white distal 8 cm., upon which laterals had not had time to develop. Branches occurred at the rate of about 8 per cm. and varied in length from 1 to 2.5 cm. Secondary branches were only 1 to 3 mm. long and occurred sparsely and only on the longest primary branches (fig. 7).

DRY CULTURE.—The root system was very much like that in the moist soil but more abbreviated. Its extent was determined by the depth of the moist soil which varied from 16 cm. on one side of the container to 35 on the other. None occurred in the surface 9 cm. (fig. 8). The dwarfed plants had fewer roots (27 and 51 respectively on two of the largest plants) than in moist soil but they were much more densely branched, *e.g.* 10–35 laterals

per cm., the longer ones having branches of the second order. The branches extended quite to the root tips where, owing to dry soil, further elongation was impossible. Where the tips had died, the roots gave rise to several long branches. In diameter, the main roots more nearly approached those of the moist soil than the thick roots of the wet soil (*i.e.*, they were mostly 1 mm. or less). They were more yellowish in color; root hairs were much more abun-

FIG. 8. Root system of *Typha* grown in dry soil. Short branches and root hairs are extremely abundant.

dant than in the drained soil, and, judging from the holding of the soil particles when excavated, more abundant also than in the moist culture. The root branches pursued markedly devious courses, in striking contrast to those in the wet and drained soils. One rhizome died without producing any growth, and another after some of its roots were only 10 cm. long.

Résumé.—A survey of the preceding data shows that decreasing aeration on the one hand and decreasing water content on the other, has a marked effect upon root habit. In moist soil, the root system was uniformly developed throughout, spreading downward and outward below the level of the rhizome to a depth of 40–53 cm. In dry soil a similar root habit was found, although the number of roots on the dwarfed plants was fewer and root-hair development was more profuse and occurred to the root ends, whose further elongation was limited by the dry soil.

Where aeration was somewhat deficient in the alternately saturated and drained soil, the root system was somewhat shallower and differentiated into two distinct parts, one of these containing two types of roots. The surface root system consisted of a network of long, fine, profusely branched roots abundantly supplied with root hairs. This was confined to the surface 9-cm. layer of soil which was best aerated. The deeper part comprised both coarse and fine main roots, the latter only being much branched.

Where plants were grown in standing water, about half of the root system grew upward and developed into water roots. These were so fine and their primary branches so abundant that for their volume they presented a large absorbing surface for dissolved oxygen. They were destitute of root hairs. Similar root development has been observed in undrained marshes. The soil roots penetrated less deeply (30 cm.) than in the drained soil but were of the two types described for the latter. The chief differences were fewer, finer, and shorter branches that were more irregularly distributed, and a relative scarcity of root hairs.

SCIRPUS VALIDUS

Scirpus made a good growth even in the dry soil. Growth was again, with one exception, in direct proportion to water content as shown in table II.

TABLE II

GROWTH OF *Scirpus validus* ABOVE GROUND WITH DIFFERENT MOISTURE CONDITIONS

CONDITION FOR GROWTH→	SATURATED SOIL	DRAINED SOIL	MOIST SOIL	DRY SOIL
Total number of stems	47.0	42.0	25.0	18.0
Average height, cm.	87.9	100.4	71.0	49.6
Average basal diameter, mm.	8.1	7.8	5.2	4.7
Dry weight of tops, grams	39.6	42.4	18.8	7.0

The panicles of the aerated (drained) culture were more abundant and further developed than those in the saturated soil. This accounts for the greater height and also for the greater dry weight. No flowers had formed

in either of the other cultures, the tips of about one-third of the plants in each being dead. The ratio of dry weight of tops to roots was just the opposite of that of *Typha*, being 5.5 to 1 for the saturated soil and 7.4 to 1 in the drained soil.

SATURATED CULTURE.—At the time of root excavation, on May 29, the water-logged soil of the wet container had a distinctly sour odor. About
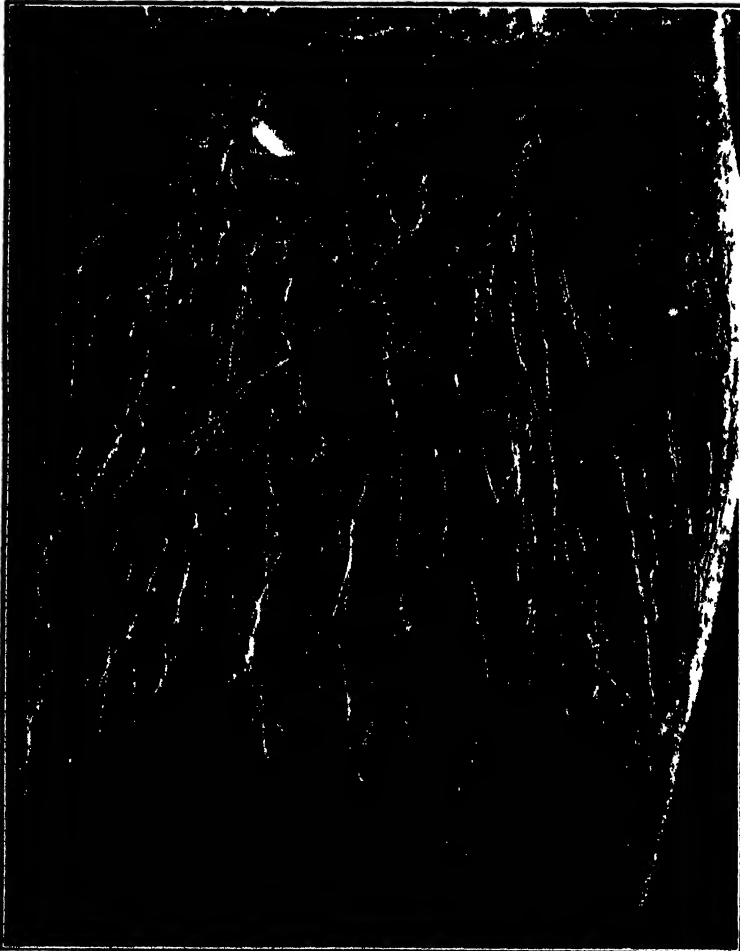

FIG. 9. Root development of *Scirpus validus* in water-logged soil covered with 5 cm. of standing water. The general root depth is about 38 cm.

half of the original rhizomes were still firm, the rest had decayed. The root system consisted of a glistening white mass of long fibrous roots that rather fully ramified the soil (fig. 9). All of these originated from the bases of

the new shoots or from new rhizomes and none from the old ones except from the part directly below the shoot. Roots were abundant to a depth of 38 cm. and a maximum depth of 46 cm. was attained. As in the case of the cattails, the horizontal and obliquely ascending roots of the surface soil were much finer and very much more branched than the deeper ones. While most of these originated from the upper portions of the nodes, the roots were not sufficiently distinct to separate into groups as in the cattails. Although a few water roots 4 to 5 cm. long were formed, a week after transplanting, this portion of the root system failed to develop. The network of fine surface roots with extremely fine laterals occurring at the rate of 3–14 per cm., developed only in the surface 2 or 3 cm. of soil. The coarser, deeper roots, 1 mm. or less in diameter, were usually sparingly branched for $\frac{1}{3}$ to $\frac{2}{3}$ of their length. The branches were simple and rarely exceeded a cm. in length. The laterals varied from 1 to 9 per cm. and in general they decreased with depth. While the main roots throughout the container were sparingly furnished with root hairs, most of the branches had none.

DRAINED CULTURE.—All of the rhizomes in the drained, unsoured soil remained firm and undecayed. As in the saturated soil, new rhizomes had made considerable growth, some a maximum of 7 cm. The root habit differed from the preceding in the following respects: the surface portion was very poorly developed, the deeper roots pursued a less devious course, and the depth of penetration was greater (fig. 10). Although some roots extended above their point of origin at the base of the shoot or rhizome, they were mostly short (10 cm. or less) and much less abundant than in the saturated soil. In fact, the surface 5 cm. of soil at a little distance from the base of the plant was only sparsely threaded with these fine roots.

In the deeper soil, the general root mass extended to 50 cm., roots were numerous at 55 cm. and a maximum depth of 59 cm. was attained. The younger roots usually reached lengths of 15 to 20 cm. before branching began and they possessed few or no root hairs. The older roots were usually branched throughout their proximal half and not infrequently almost to their tips. The branches were more numerous on the older roots, the number and length of branches being practically the same as that recorded for the saturated soil.

MOIST CULTURE.—The root system in the moist soil was much deeper than under either of the preceding conditions. Roots were very numerous at 64 cm. depth and a maximum depth of 71 cm. was attained. They originated only from the portion of the rhizome adjacent to the growing shoot, or from the base of the shoot. The soil below the level of the rhizomes was filled with a mass of roots, although they were not so abundant as in the drained soil. In general, their course was rather directly downward. Many main

roots in the upper soil were 10 cm. or less in length and the tips were dead. Short laterals, rarely exceeding 9 cm. in length, occurred regularly at the rate of 9–14 per cm. throughout the length of the main roots, except on the youngest 5–10 cm. of the root-ends. It appeared that the extremely abun-
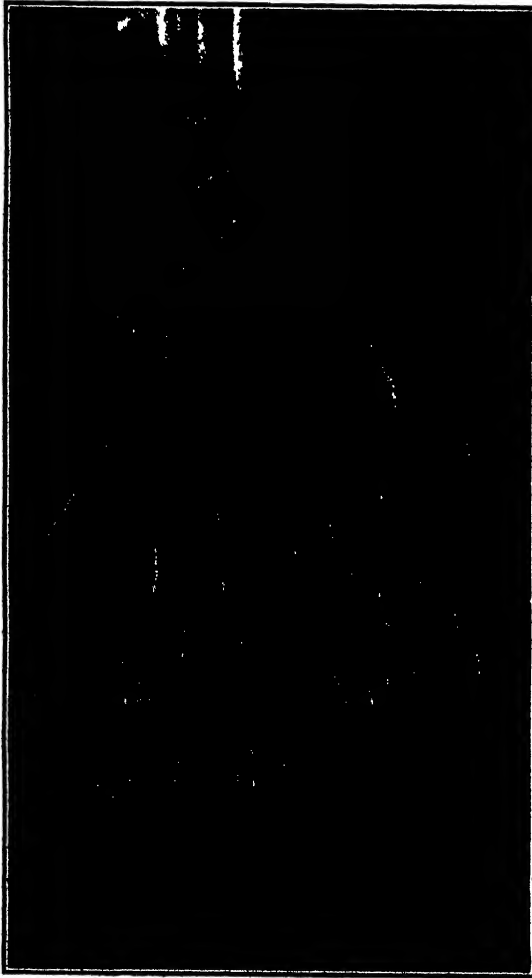

FIG. 10. Root system of *Scirpus* in drained soil (depth about 55 cm). The development of a surface root system is much less marked than in figure 9.

dant roots were functioning throughout their entire course. Both the main roots and their branches were thickly covered with long root hairs. Portions of all of the old rhizomes showed decay and some were completely rotted.

DRY CULTURE.—The root systems in the dry soil were very much abbreviated as were also the tops. Roots were not only shorter but also fewer per plant, although more abundant in proportion to tops than in the wet soil. Insufficient water was clearly the limiting factor to growth since there was an abundant supply of food in the fairly well preserved rhizomes. As in the moist culture, there were many roots that extended horizontally or obliquely downward into the shallower soil but in no case were they found approaching the soil surface. The general direction of root growth was vertically downward or obliquely outward and then downward. The general level of root penetration was 30 cm., a few penetrating 10 cm. deeper. The older, deeper roots were profusely branched throughout but scarcely more so than in the moist container. Moreover, the branches were shorter, averaging only 3 mm. Since the main roots had ceased elongating, branches occurred to within 3 or 4 cm. of the tip. Although none of the primary laterals were rebranched, both they and the main roots were more densely clothed with root hairs than were those in the moist soil. Moreover, both branches and root hairs extended quite to the root tips. Hence, as shown in figure 11, the soil was held tenaciously.

RÉSUMÉ.—A comparison of the root behavior under the different environments shows, as in *Typha*, that under conditions of poor aeration there is a development of a fine, much branched network of surface roots and a consequent decrease in depth of penetration. The bulrush seems to emphasize the surface absorbing system somewhat less than the cattail. No water root system was formed and the surface system was less extensive, being confined to the first 3 cm. of soil. Moreover, in the drained soil it was very poorly developed. Here the deeper roots were straighter and penetrated about 12 cm. farther. Under both conditions branching was sparse and root hair development poor. In both the well aerated moist and dry soils, the surface root system failed to develop. Roots were 14 cm. deeper in the moist soil than in the drained, branching was quite profuse, and root hairs were abundant throughout. Relative to size of tops, the dry soil had the most roots. Their limited extent was compensated in part by the profuse development of short branches to the tips and by the very marked development of root hairs.

PHRAGMITES COMMUNIS

Phragmites made the poorest growth of all four species, a fact undoubtedly due to its being transplanted after growth began. The tops decreased in average height from 101 cm. in the saturated soil to 8 cm. in the dry soil where very little growth occurred. Total leaf production ranged in the same sequence from 61 leaves to only 4, notwithstanding the fact that the dry culture had the largest number of stems. Leaf area ranged likewise

from 1,118 to 20 square cm., that of the drained soil exceeding the saturated. The dry weight of tops of the drained soil was also somewhat greater. The ratio of tops to roots was less in the drained than in the saturated soil.

SATURATED CULTURE.—When the container was opened for root study on June 3 it was found that the soil had a distinctly sour odor but none of the rhizomes were decayed. The root system was composed of two distinct parts, a very much branched surface system and a portion that would ultimately penetrate deeply. The roots of the latter generally originated from
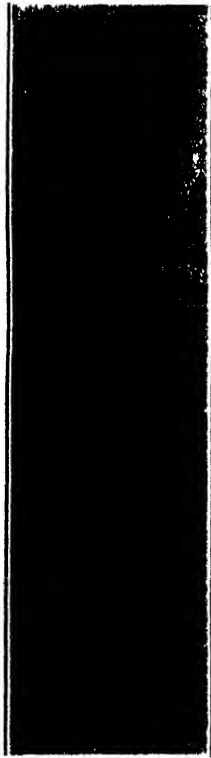
FIG. 11. Main roots of *Scirpus* grown in dry soil. Branches and root hairs occur to the root-tips.

the base of the rhizome branches, those of the former on the higher nodes of the shoot. Because of the repeated branching of the rhizomes at different levels, horizontal roots often originated more deeply than those that penetrated more or less vertically.

Some of the roots of the surface system arose as deep as 14 cm. and the surface soil was filled to its contact with the water with a mass of shining white rootlets. All were a mm. or less in thickness and none exceeded 28

cm. in length. While the younger ones often had unbranched ends several centimeters long, the older ones were profusely branched nearly to their tips. The branches were so very numerous (30 to 85 per cm., although only 0.5 to 10 cm. long and poorly rebranched) that the total surface for the absorption of water and oxygen was greatly increased.

The deeper roots had twice the diameter of the shallow ones. They extended to a depth of 21 cm. Only a few primary branches occurred and they were on the oldest part. Both main roots and branches of the entire root system were destitute of root hairs, except on a relatively small portion of the older ones.

DRAINED CULTURE.—The soil in this container was also sour but none of the rhizomes were decayed. With one exception, the depth of penetration was the same as before, but the following differences were plainly evident. The surface root system, although well developed, was not nearly so pronounced as in the saturated soil. Primary laterals on the entire root system were finer, longer, more numerous on the deeper parts, and more profusely branched, but rarely to the second order. Root hairs were abundant throughout the soil mass on both the main roots and practically all of the branches.

MOIST CULTURE.—The root system had made a poor growth in this container as had also the tops. Two rhizomes had died, one had not developed, and another had developed three short aerial shoots but no roots. The horizontal, surface root system consisted of wire-like, profusely branched roots, some occurring at a depth of 15 cm. That they were young was indicated not only by the absence of branches near the root-ends but also by the fact that approximately half of their laterals were simple. Moreover, they were not abundant. Of the few roots that penetrated downward, all but two were less than 16 cm. in length; the longest extended 34 to 37 cm. Root hairs were abundant throughout.

DRY CULTURE.—The poor growth showed that even the surface soil in this container was too dry for much development. Two rhizomes had decayed, three had developed short, erect stems but no roots, one had rooted and died. The others had from 3 to 6 roots each that varied from 1 to 20 cm. in length, but they were mostly less than 10 cm. The two types of roots were in evidence but neither was as well developed as in the preceding container.

RÉSUMÉ.—In the saturated soil, *Phragmites* developed a network of fine, extremely well branched roots which filled the surface 10 cm. to its contact with the water. The coarser, more or less vertically penetrating roots extended to about twice this depth and branched but little. There were no root hairs except on a small portion of the oldest roots. In the better

aerated, drained soil the root habit differed only in a less pronounced development of the surface root system and in the fact that root hairs were abundant on practically all of the main roots and their branches. In well aerated, moist soil the surface root system was represented by only a few horizontal roots, some of those of the deeper portion reaching nearly twice the depth attained in the poorly aerated culture. Few roots grew in the dry soil.

SPARTINA MICHAUXIANA

Spartina made a rapid growth, the plants in the saturated and drained cultures both reaching an average height of 75 cm., in comparison to 55 and 35 respectively for the drier soils. Dry weight of tops again decreased directly in the sequence of decreasing water content of soil except that the moist soil produced slightly more dry matter than the drained. The top-root ratios of the saturated and drained soils were 8.2 to 1 and 5.6 to 1 respectively.

SATURATED CULTURE.—The root system was more or less differentiated into a shallower and a deeper portion. The former consisted of a large number of fine, profusely branched, horizontally placed roots, some 20 cm. in length, that ramified throughout the shallower soil extending to its surface. Many of these ended without turning downward. They were branched abundantly with long laterals but root hairs were very sparse.

The deeper portion of the root system was chiefly composed of numerous coarse roots nearly 2 mm. thick that extended vertically downward or ran obliquely downward with little change in the direction of their course. Many ended at 28 cm. depth, a few extended 5 to 10 cm. deeper. Some of the shining white younger roots were only 10 cm. long, and entirely unbranched but sparsely clothed with root hairs. The last 10 cm. of the older roots was likewise frequently free from branches. Otherwise branching occurred at the rate of 9–16 laterals throughout. These varied in length from 1 to 10 cm. Root hairs were sparse and found on only relatively few branches. Short secondary branches occurred only on the longer laterals. Another lot of the deeper roots was much finer and could not be distinguished except by position from the fine surface roots. They were evidently older than those just described. All were abundantly clothed with fine laterals usually 0.5 to 3 cm. in length. Only the longer ones were re-branched and root hairs were sparse. Many new rhizomes had originated from the base of the clumps, but none exceeded 7 cm. in length.

DRAINED CULTURE.—The development of the rhizomes and general extent of the root system were very similar to those of the saturated soil, general root extent being only 5 cm. deeper. The surface portion of the root system was the same as in the previous culture but in neither were the roots densely

matted as in *Typha* and *Scirpus*. Not only was the rate of branching some-
what increased but also the primary branches averaged twice as long and
fairly extensive laterals occurred even on the shorter rootlets. The roots
were branched more nearly to their tips. Moreover, root hairs were much
more abundant than in the saturated soil. Thus, the effects of increased
aeration were clearly apparent.

MOIST CULTURE.—Quite in contrast to the abbreviated root system of the
wet soil, 90 per cent. of the roots extended to the bottom of the container,
thus having a length of nearly 90 cm. Moreover, none were found in the
surface soil, all penetrating vertically or obliquely downward. Many of the
coarse, rapidly growing roots were 2 mm. thick and the distal 15–25 cm. were
unbranched. There was a decrease in thickness to approximately 0.5 mm.
on older roots, and an increase in the degree of branching, long branches
occurring close to the root-ends. Laterals occurred at the rate of 6–14 per
cm.; branches exceeding 10 cm. in length were not abundant and most were
shorter. The longest laterals (20–30 cm.) were found in the last 20 cm. of
soil. Secondary laterals were common and root hairs abundant throughout.
Where the roots entered the coarse gravel at the bottom of the container,
their diameter was doubled and the branches were much coarser.

DRY CULTURE.—The roots had penetrated quite beyond the 20 cm. of
moist soil and through the dry soil to the bottom of the container at 58 cm.
In one case this was true of 14 roots from a single rhizome. In many cases
they extended 5–14 cm. along the bottom of the container and gave off great
clusters of long branches very nearly to their tips.

The roots were distinctly smaller in diameter than those in the wet soil,
none exceeding a mm. and some were only $\frac{1}{3}$ as large. This was in striking
contrast to the thick, rapidly growing roots of the moist soil.

All of the numerous coarse roots ran either vertically or obliquely down-
ward so that there were none in the surface 6 cm. of soil. Branching was
very similar on all of the roots, being uniform and profuse throughout the
soil mass. It was somewhat more profuse than in the moist culture, and
many branches, especially near the root-ends, were longer. Branches oc-
curred at the rate of 9 to 22 laterals per cm. on both main roots and primary
laterals. The primary laterals usually extended out horizontally 2–4 cm.,
although many, especially near the root-ends, were longer. Branches of the
third order were not infrequent, all of the smaller branches being very fine.

RÉSUMÉ.—*Spartina* reached the same height in saturated and drained
soils. Decrease in dry weight of tops accompanied decrease in water con-
tent, except that in the moist soil dry weight was slightly greater than in
the drained.

The grass responded to poor aeration by the development of numerous
fine, long, well branched horizontal roots extending to the soil surface. This

part of the root system was less poorly developed than in either *Typha* or *Scirpus*. The deeper portion of the root system was only slightly more extensive in the drained soil, but here it was furnished with more and longer branches. Root hairs were sparse in water-logged soil but more abundant in the drained.

No surface aerating roots developed under good aeration in moist soil, but the others grew to almost three times the depth attained in the intermittently water-logged soil. Branches were more numerous and root hairs very abundant throughout. Roots and branches in the coarse, well aerated gravel, were of much greater diameter. In the drier culture the roots penetrated soil of only 2 per cent. available water content, their extent being limited by the size of the container. Branching was most profuse here and the number of branch orders greatest. Root hairs were much more abundant also than in the moist soil.

Discussion

Of the four species investigated, three are characteristic of the reed-swamp stage of the hydrosere, all being very widely distributed. *Scirpus* grows in the deepest water, sometimes in excess of 6 feet, and *Typha* in shallower places. *Phragmites* usually occupies wet areas which are submerged at least for a part of the growing season, while *Spartina* represents a very late stage of the hydrosere and forms a transition between swamp plants and more mesophytic vegetation. Owing to changes in water level resulting from drought, flooding, and other causes, the above sequence is not absolute and relicts of cattails and bulrush often occur intermixed with the reed. In fact, the great extremes of water and air content endured by the plants, once they are thoroughly established, are indeed remarkable. *Spartina*, in these experiments for example, flourished in water-logged soil and also, after growth was started, made a fair development in soil with only 2 per cent. available water content.

These experiments show clearly that plants growing naturally in poorly drained and poorly aerated habitats are much less sensitive to the composition or absence of a soil atmosphere than are those in naturally well drained soils. In water-logged soil the oxygen supply must be very low or practically nil. Oxygen diffuses slowly through water, so that the supply is not quickly replenished by diffusion alone. The addition of tap water through a sprinkler to that standing on the soil surface together with the photosynthetic activity of the algae considerably increase the supply of dissolved oxygen. It has been demonstrated by several investigators that algae may bring about the supersaturation of lakes and streams as a result of photosynthesis, at the same time preventing the accumulation of large amounts of carbon dioxide, in consequence of the same process (3, 13).

All four species showed a distinct aerotropic response by the development of a superficial root system with a very extensive absorbing area. *Typha* was the only species, however, that extended its roots into the water layer above the soil. This development of an aerating portion of the root system and its absence under conditions of good aeration is in accordance with the findings of WILSON (26). He observed that the number and size of "knees" of the bald cypress (*Taxodium distichum*) were determined by the height of the water and the duration of the flooding. Young roots often grew directly upward until they reached the surface when they again turned and developed beneath the water. In dry soil the trees showed no trace of such development. Similar structures occur about the bases of trunks of Tupelo gum (*Nyssa aquatica*) in swamps, the roots bending sharply upward to a distance of 6 to 8 inches above the surface of the water and then turning downward into it again.

The production of adventitious roots which run horizontally above oxygen-free swamp soil, as in *Alnus, Fraxinus*, and various other land species, is well known (cf. 8). But less exact information is available on the root behavior of true hydrophytes, although upright roots have been developed on *Rumex* and *Nymphaea* when these were planted too deeply (11).

In considering root response to aeration it must be kept in mind that species vary widely, a substratum of water or water-logged soil may furnish sufficient oxygen for certain hydrophytes, while under similar conditions most plants would succumb. CANNON (4) has shown that the temperature relation is of great importance in problems of aeration, the species studied demanding better root aeration as the soil temperature became higher. Since these cultures were grown under a moderate and rather uniform soil temperature (about 70° F.) the temperature factor need not be considered. Although there is some evidence that the rate of root respiration in hydrophytes is lower than that of land plants (10), the capacity of marsh plants to grow in water-logged soil is undoubtedly due to their extensive development of aerenchyma in leaves, stems, and roots (25). The continuously open stomata in many of them (*e.g., Typha* and *Scirpus*) would facilitate rapid gaseous diffusion. The extent to which aerenchyma develops when the roots of grasses like *Spartina* are continuously submerged remains to be determined, although a marked increase of intercellular spaces in roots of corn, etc., under such conditions has been clearly shown (20). The dependence of root-hair development of various plants upon the presence of oxygen has been pointed out by several investigators (6, 23). Although water roots are commonly without hairs, they may readily be produced on some plants in an aqueous medium provided that calcium is present (9). Root elongation in nearly all plants is likewise retarded by a deficient oxygen supply.

Summary

Plants of great bulrush (*Scirpus validus*), cattail (*Typha latifolia*), reed (*Phragmites communis*), and tall marsh grass (*Spartina michauxiana*) were grown in large containers under four sets of conditions of aeration and water content, *viz.*; water-logged soil, soil alternately saturated and drained, moist soil, and dry soil.

Growth of tops in *Typha,* as measured by number and size of leaves and total dry weight, increased with increasing water content until a condition of saturation was reached.   More and larger *Scirpus* grew in the saturated soil but because of earlier flower-stalk production in the drained soil, both height growth and dry weight of tops were greatest in that culture.   Both *Phragmites* and *Spartina* grew about equally well in the water-logged and drained soils but like *Typha* and *Scirpus* they became progressively poorer as the soil became drier.

The ratio of tops to roots (based on dry weight) was less in the drained than in the water-logged soil, except in *Scirpus.*

Typha alone had roots that developed in water.   These were of small diameter and possessed so many fine laterals that they presented a large surface for the absorption of dissolved oxygen.

A response to poor aeration was shown in all four species by the development of a shallow root system of fine, much branched roots which presented a large surface area in proportion to volume.   This did not develop in well aerated soil, except to a limited extent in *Phragmites.*

Aside from this surface portion of the root system, the development of lateral roots increased with an increase in aeration.   Where dry soil hindered or prevented root elongation, branching occurred to the root tips.

Root depth increased with decreasing water content until the soil became too dry for root growth.

Root hairs were absent in water, few and irregularly distributed in saturated soil, but progressively more abundant as aeration became better with decreasing water content.

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LITERATURE CITED

1. ANDREWS, F. M., and BEALS, C. C.   The effect of soaking in water and aeration on the growth of *Zea mays.*   Bull. Torrey Bot. Club **46**: 91–100. 1919.
2. BALLS, W. L.   The cotton plant in Egypt, studies in physiology and genetics.   Macmillan & Co., Ltd., London. 1919.
3. BERGMAN, H. F.   The relation of aeration to the growth and activity of roots and its influence on the ecesis of plants in swamps.   Ann. Bot. **34**: 13–33. 1920.

4. CANNON, W. A. The influence of the temperature of the soil on the relation of roots to oxygen. Science, n.s. **58**: 331–332. 1923.

5. ——————. Physiological features of roots, with especial reference to the relation of roots to aeration of the soil. Carnegie Inst. Washington, Pub. No. 368. 1925.

6. ——————, and FREE, E. E. Root adaptation to deficient soil aeration. Carnegie Inst. Washington, Year Book **19**: 62. 1920.

7. CLEMENTS, F. E. Aeration and air-content; the rôle of oxygen in root activity. Carnegie Inst. Washington, Pub. No. 315. 1921.

8. EMERSON, F. W. Subterranean organs of bog plants. Bot. Gaz. **72**: 359–374. 1921.

9. FARR, C. H. Studies on the growth of root hairs in solutions. Amer. Jour. Bot. **14**: 446–456. 1927.

10. FREYBERG, E., and MAYER, A. Ueber die Athmungsgrösse bei Sumpf- und Wasserpflanzen. Landw. Versuchs-St. **23**: 463–470. 1879.

11. GOEBEL, K. Ueber die Luftwurzeln von *Sonneratia*. Ber. deutsch. bot. Ges. **4**: 249–255. 1886.

12. HALL, A. D., BRENCHLEY, W. E., and UNDERWOOD, L. M. The soil solution and the mineral constituents of the soil. Phil. Trans. Roy. Soc. London, B. **204**: 179–200. 1913. Jour. Agr. Sci. **6**: 278–301. 1914.

13. HARRISON, W. H., and AIYER, P. A. S. The gases of swamp rice soils—II. Their utilization for the roots of the crop. Mem. Dept. India, Chem. Ser. **4**: 1–17. 1914.

14. HOLE, R. S. Recent investigations on soil-aeration. Part II. With special reference to forestry. Agr. Jour. India **13**: 430–440. 1918.

15. HOWARD, A. Soil aeration in agriculture. Agr. Res. Inst., Pusa. Bull. 61. 1916.

16. ——————, and HOWARD, G. L. C. Soil ventilation. Agr. Res. Inst., Pusa, Bull. 52. 1915.

17. ——————, ——————. Drainage and crop production in India. Agr. Jour. India **14**: 377–387. 1919.

18. ——————, ——————. Some aspects of the indigo industry in Bihar. Dept. Agr. India, Mem. Bot. Ser. **11**: 27–36. 1920.

19. HUNTER, C. Some observations on the effects of soil aeration on plant growth. Proc. Univ. Durham Phil. Soc. **4**: 183–186. 1912.

20. ——————. The aerating system of *Vicia faba*. Ann. Bot. **29**: 627–634. 1915.

21. KNIGHT, R. C. The response of plants in soil- and in water-culture to aeration of the roots. Ann. Bot. **38**: 305–325. 1924.

22. LIVINGSTON, B. E., and FREE, E. E.   The effect of deficient soil oxygen
    on the roots of higher plants.   The Johns Hopkins Univ. Circ. n.s.
    3: 182–185.   1917.
23. SNOW, L. M.   The development of root hairs.   Bot. Gaz. 40: 12–48.
    1905.
24. WEAVER, J. E., and CLEMENTS, F. E.   Plant ecology.   McGraw-Hill
    Book Co., New York.   1929.   (p. 321.)
25. Ibid. (p. 345.)
26. WILSON, W. P.   The production of aerating organs on the roots of
    swamp and other plants.   Proc. Acad. Nat. Sci. Phil. 1889: 67–69.
    1889.   Bot. Cent. 43: 148.   1890.

A FURFURAL–YIELDING SUBSTANCE AS A SPLITTING PRODUCT OF PROTOPECTIN DURING THE RIPENING OF FRUITS[1]

C. M. CONRAD

Introduction

While studying the CARRÉ (4) procedure for the determination of protopectin, the writer observed that the filtrates from the calcium pectates contained a considerable quantity of a furfural-yielding substance. At about the same time EHRLICH's (9) preliminary paper came to the writer's attention. In this paper EHRLICH showed that the protopectin (he calls it "pectin") of sugar beets and a considerable number of other vegetables and fruits is split by boiling water into two fractions—"a levo-rotatory araban," and "a calcium-magnesium salt of pectic acid." A mixture of the two, called "hydrato-pectin" is obtained by boiling the plant tissues, previously freed from sugars, with water and evaporating the extract to dryness on the water bath. The araban is then separated from the pectic substances by extraction with 70 per cent. alcohol. The work of EHRLICH (11) on sugar beets has been confirmed, in general, by SMOLENSKI (20) and his co-workers.

The exact nature and significance of EHRLICH's work seems not to be very well appreciated by other investigators in this field. For example, McKINNIS (16) apparently fails to distinguish between the extraneous "araban," soluble in 70 per cent. alcohol, and the arabinose contained within the pectin molecule (17). It is likewise not clear from DORE's (8) discussion that he distinguished between the two. WICKMANN (21) found a furfural-forming complex in his pectic acid filtrates but ascribed it to disintegration of pectin. In spite of EHRLICH's work, EMMETT (12) assumes that no alcohol-soluble substance forms from the insoluble cell wall material of pears during ripening. However, she is at a loss to explain a certain unidentified constituent in the juice which makes up 20 to 30 per cent. of the dry weight.

It should be noted that EHRLICH uses a different nomenclature from that generally used, and from that proposed by the Committee (6) on Nomenclature of Pectic Substances. Thus his "pectin" corresponds to the protopectin of that committee report and his "calcium-magnesium salt of pectic acid" to the pectin. This unfortunate difference in terminology is, no doubt, largely responsible for the present confusion and the failure to draw correct deductions from EHRLICH's work.

[1] Approved for publication by the Director of the Experiment Station.

By boiling the tissue with water, EHRLICH brought about a splitting of beet and flax protopectin into pectin and a polysaccharide. Allowing for the different nomenclature he assumed the following reaction:

$$\text{protopectin} + H_2O \longrightarrow \text{pectin} + \text{polysaccharide}$$
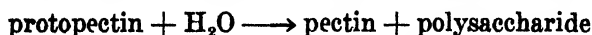
The polysaccharide was composed of pentose only (sugar beet) or of pentose and hexose (flax stems, 10). Since pectin increases naturally (2) during the ripening of many fruits, it was the purpose of this investigation to determine whether a pentose-bearing polysaccharide is liberated at the same time. A reaction taking place according to the above equation should show a definite relation between the amounts of pectin and polysaccharides for all stages of ripeness. Then by determining the furfural yield and noting whether or not the ratio of furfural to pectin is a constant it should be possible to test the correctness of the equation.

Preliminary experiments showed that the alcoholic extracts from ripe apples, bananas, and pears contained a considerable quantity of a furfural-yielding substance. A sample of Stayman apples contained substances soluble in 70 per cent. alcohol which yielded 0.67 per cent. of furfural based on dry weight. After acid splitting of the protopectin and extraction with 70 per cent. alcohol the same sample gave 0.57 per cent. additional furfural. When these values are calculated as pentose, they are equivalent to approximately one half the amount of the pectic substance. As neither unripe apples nor pears were available when the work was undertaken they were not studied in the present series. The first report deals with the occurrence of this alcohol-soluble furfural-yielding substance, and its relation to the pectic substances in several fruits during ripening. A later report will deal with the nature of the furfural-yielding substance.

Description of experimental material

The fruits studied were bananas, peaches, strawberries, and dewberries. They were either picked in different stages of ripeness, or taken at an unripe stage and allowed to ripen in a controlled temperature chamber at 30° C. This temperature had been found by previous work to bring about rapid pectic changes. The stage of ripeness was selected on the basis of color and softness.

BANANAS

Forty medium sized fruits were carefully cut from a bunch of green bananas and assorted into four lots of equal number and approximate weight. Lot I was sampled at once according to methods to be described. In this lot the fruits were so green that the skins could not be stripped off. The remaining lots were placed in the constant temperature chamber at

30° C. Lot II was removed after 2 days. The skins had yellowed considerably but the fruit was not ripe enough for eating. Lot III was removed after 7 days. The aroma was very noticeable and the condition excellent for the table. Lot IV was removed after 11 days. The skins had become dark and the fruits were in an overripe condition for the table.

PEACHES

Early Georgia peaches, still firm to the touch, were obtained on the market. Four uniform lots of eight fruits each were selected. Lot I was sampled at once. The remaining lots were stored at 30° C. Lot II was removed after 19 hours. The flesh of the fruit was perceptibly softer than that of lot I. Lot III was removed after 46 hours. The flesh had become quite soft. Lot IV was removed after 72 hours. The skins were yellow, the flesh was very soft, and a marked aroma was present.

STRAWBERRIES

The variety Klondike, grown on the Experiment Station farm, was used for this experiment. Berries in three stages of ripeness were picked at the same time and a lot selected from each. Lot I contained fruits turning white just before coloring. The berries of lot II had colored over about half the surface. Lots III and IV were at the same stage and full ripe. Lot IV differed from lot III only in being stored for 47 hours at 30° C. During this period the berries had increased in color, withered considerably, and lost about 14 per cent. of their weight.

DEWBERRIES

The berries in this case grew wild a short distance from the Station farm. Two lots were picked at the same time. In lot I the berries were just beginning to turn and were still hard to the touch. The berries of lot II were full ripe and soft.

Preparation and storage of the samples

Since the furfural-yielding substance was to be separated with 70 per cent. alcohol, and since this concentration is excellent for preservation, it was found convenient to use this medium for the storage of the samples. In case the fruit was ripened artificially the loss of weight during this process was determined. The weights of the pits in the peaches were noted and the loss of weight of this fruit was calculated as loss in the flesh only. The fruits were cleaned, pulped by means of a Nixtamal mill, and 100-gram samples of the pulp weighed out into 500-cc. Erlenmeyer flasks. The samples were covered with the calculated amount of 95 per cent. alcohol to give a final concentration of 70 per cent. by volume. At the same time moisture samples were taken to permit calculation to dry weight.

Separation and treatment of the different fractions

First, the furfural-yielding substance, soluble in 70 per cent. alcohol, was separated from the pectic substances by exhaustive extraction of the pulp. The storage alcohol was removed through a hardened paper on a Buchner funnel. The drained pulp was then washed with 200 cc. of 70 per cent. alcohol, added in successive 50-cc. portions. The pulp was drained by sucking as dry as possible before the addition of each new portion of alcohol. It was next transferred to a shaking bottle with 200 cc. of 70 per cent. alcohol and shaken for 30 minutes. This alcohol was drained off and the pulp again washed with 200 cc. of 70 per cent. alcohol, added in portions. The pulp, thus treated, contained no more furfural-yielding substance soluble in 70 per cent. alcohol until subsequently treated to decompose the protopectin. It was washed with two portions each of 95 per cent. alcohol and ether and dried at 30° C.

The free pectin, which was insoluble in 70 per cent. alcohol, was now extracted. The dried residues were first shaken an hour with 500 cc. of 0.2 per cent. ammonium citrate solution at laboratory temperature. The purpose of the ammonium citrate was to dissolve any pectic acid which might have been formed from the pectin by pectase activity. A previous experiment showed that shaking for 30 minutes brings about complete solution but it was continued for an hour to make certain. The extract was filtered off through soft fluted paper and an aliquot portion used for the pectin determination. The pulp was now further washed to remove the last traces of pectin. This was accomplished by washing back into the shaking bottles with 500 cc. of water or 0.2 per cent. ammonium citrate solution, in case any pectic acid was present, and shaking 30 minutes. The extract was filtered off and discarded. This process was repeated 5 to 7 times although not more than traces of pectin were ever found after the third washing. Finally the pulp was dried as previously described with 95 per cent. alcohol and ether.

There remained in the dried residues only substances insoluble in 70 per cent. alcohol and water,—among others, protopectin. The protopectin was decomposed by covering the residues with 100 cc. of thirtieth normal hydrochloric acid (in one case 150 cc.) and heating at boiling temperature under a reflux condenser for an hour. The mixture was diluted with sufficient 95 per cent. alcohol to give a final concentration of 70 per cent. The acid was then neutralized with the calculated quantity of standard sodium hydroxide solution. The polysaccharide and the liberated pectin were again extracted with 70 per cent. alcohol and then water exactly as described for the soluble fraction. The residues were finally dried and preserved.

Chemical methods

Moisture.—The samples, consisting of 3 to 5 grams of wet pulp, were dried to constant weight at 80° C. in a vacuum oven at approximately 2.5 cm. of mercury.

Determination of furfural from the alcoholic extracts.—The combined 70 per cent. alcoholic extracts and washings, amounting to something over a liter, were evaporated in a porcelain dish on the water bath to 30–40 cc. At this point any appreciable amount of sugar in the liquid was removed by fermentation. This was found to be necessary because of the production of hydroxy-methyl-furfural (7) on distillation of hexose sugars with 12 per cent. hydrochloric acid. The presence of this furfural derivative causes the development of a dirty green color in the FLEURY and POIROT (13) method instead of the pure blue due to furfural. The sugars were fermented away with washed baker's yeast, observing the precautions suggested by ABBOTT (1). The alcoholic extracts obtained in connection with the protopectin contained inappreciable amounts of sugars and were not submitted to fermentation.

The evaporated extracts, freed from more than traces of sugar, and separated from the yeast by filtration were transferred to a 750 cc. distilling flask. This was done by washing with sufficient concentrated hydrochloric acid and water to make 200 cc. of 12 per cent. hydrochloric acid solution. The mixture was then steam distilled and the furfural collected according to the procedure of PERVIER and GORTNER (18). Distillation was continued until ten drops of the distillate gave a negligible color with aniline acetate in acetic acid solution.

Because of the small amount of furfural in most of the distillates the usual method of determining the furfural by precipitation with phloroglucinol is unsatisfactory. Neither is the electrometric titration method of PERVIER and GORTNER (18) sufficiently sensitive. The bisulphate method of JOLLES (14) was tried but known amounts of furfural could not be recovered, quantitatively. The colorimetric method of SCHAFFER (19) was not very sensitive. The colorimetric method of YOUNGBURG and PUCHER (22) proved extremely sensitive and does not give any color with hydroxy-methyl-furfural, but due to instability of the color with these distillates checks could not be obtained. Finally the colorimetric method of FLEURY and POIROT (13) was selected because of its high sensitivity and satisfactory stability of color. In this method the colors obtained from the distillate are compared with those of standards prepared from pure furfural redistilled at low pressure. The following reagents are used:

Reagent A
    Acetic acid (pure) .... 1000 cc.
    Orcine .... 0.625 gm.

Reagent B
    Hydrochloric acid (sp. gr. 1.19)...... 1000 cc.
    Ferric chloride .... 0.06 gm.
Standard furfural solution
    Furfural, pure .... 1.0 gm.
    Acetic acid .... 10.0 cc.
    Water, to make .... 1000.0 cc.

The standard furfural solution contains 1 mg. of furfural per cubic centimeter. The solution has been found to maintain its strength over several months. More dilute standards are prepared by diluting this solution with 1 per cent. acetic acid solution. The concentration of furfural was checked by the standard phloroglucinol method.

The procedure for the FLEURY and POIROT method is as follows: Place 1 cc. (quantitative pipette) of the "unknown" furfural solution in a test tube. Immediately add 4 cc. of reagent A. In a second test tube place 1 cc. of the appropriate standard solution and add 4 cc. of reagent A to this, also. Next add to the tubes at one minute intervals, 5 cc. of reagent B. Plunge the tubes into a boiling water bath containing a "false bottom" and leave exactly one minute. Remove the tubes and allow to stand 30 minutes, when the color will have reached its maximum. The color remains at a maximum for at least 30 to 40 minutes more after which it may fade slowly. The manipulations of the colorimeter suggested by McCRACKAN, PASSAMANECK, and HARMAN (15) were found to simplify calculations materially.

In using the FLEURY and POIROT method several precautions should be observed. The colors themselves cannot be diluted, so the "unknown" and standard colors should match within 20 per cent. If, on comparison, this limit is found to be exceeded a new colorimetric determination must be carried out with standard or "unknown" furfural solution diluted accordingly. Also reagent A should be made of a good grade of chemicals. The acetic acid must be free from furfural. This reagent should remain clear for a number of days. A dark solution, or one that turns dark quickly, will not give accurate color comparisons.

Pectin and pectic acid.—In bananas, this fraction was present entirely as pectic acid. In none of the other fruits was any pectic acid found. The pectic acid is assumed to have arisen from pectin through pectase activity. As stated above, the dried residues, after the first alcoholic extraction, were ground and shaken an hour with 0.2 per cent. ammonium citrate solution. After filtration, aliquots of the filtrate were taken for a determination of pectin by the CARRÉ-HAYNES (5) method. Impurities in the calcium pectates were evaluated by the method described by APPLEMAN and CONRAD (3).

Protopectin.—After complete removal of the soluble fraction with alcohol and water, as described in a previous section, the dried residues were boiled

an hour with thirtieth normal hydrochloric acid. This converted the proto-pectin into pectin and liberated more of the alcohol-soluble furfural-yielding substance. After separation of the furfural-yielding substance the residue was extracted with 0.2 per cent. ammonium citrate solution, or water, and the pectin determined as calcium pectate.

Experimental results

The results of the different experiments are brought together in table I.

As was to be expected, pectin increases and protopectin decreases during ripening. While the sum of the pectin and protopectin fractions is not en-tirely constant for the different stages of ripeness it nevertheless approaches a constant value and the discrepancy is possibly within the limit of the experimental error. Concurrently with the increase in pectin, there is an increase in polysaccharide as represented by furfural yield. The ratio of furfural to calcium pectate (column six) shows that the increase in poly-saccharide is practically proportional to the increase in pectin, although this ratio seems to vary with the different fruits. In the protopectin fraction as protopectin decreases the polysaccharide decreases. Here, however, the ratio of furfural to calcium pectate is higher and fluctuates more, especially with bananas. A partial explanation for the higher ratios and greater fluctuation will be discussed in a later paragraph. The totals of calcium pectate and of furfural show a tendency either to increase or decrease pro-gressively during ripening. This same tendency is shown by the ratios of the totals. However, the ratios here are more nearly the same for the dif-ferent fruits.

Discussion of results

Inspection of the ratios of furfural to the pectic substances reveals a rather close relationship. Thus during the ripening of the different fruits, pectin and polysaccharide exist free in the sap in an approximately fixed proportion. However, this ratio varies from one fruit to another. It is possible that the protopectin itself may differ slightly in the different fruits, either in containing a greater proportion of polysaccharide or in containing a polysaccharide with a greater number of pentose units. The ratios in the protopectin fraction are, in general, higher. In bananas the ratio fluctuates greatly. However, the banana was the only fruit to show strong pectase activity and it is quite possible that pectinase existed here also and destroyed part of the pectin so that it was not determined. The high ratios of lots III and IV are undoubtedly due to low pectin figures and not to large amounts of polysaccharide.

A factor that makes all the ratios in the protopectin fraction higher than those of the soluble fraction is the destruction of pectin that accompanies

TABLE I

ANALYSIS OF FRUITS BASED ON ORIGINAL FRESH WEIGHT

T NO.	LOSS ON STORING	MOISTURE	SOLUBLE IN THE FRUIT AS SAMPLED			FROM THE PROTOPECTIN BY SPLITTING WITH DILUTE ACID			TOTALS BY SUMMATION OF THE SOLUBLE AND INSOLUBLE FRACTIONS		
			POLYSACCHARIDE AS FURFURAL	PECTIN AS CA-PECTATE	RATIO: FURFURAL/CA-PECTATE	POLYSACCHARIDE AS FURFURAL	PECTIN AS CA-PECTATE	RATIO: FURFURAL/CA-PECTATE	POLYSACCHARIDE AS FURFURAL	PECTIN AS CA-PECTATE	RATIO: FURFURAL/CA-PECTATE
	per cent.	per cent.	per cent.	per cent.		per cent.	per cent.		per cent.	per cent.	
BANANAS											
I	0.0	72.75	0.003	0.106	0.03	0.015	0.201	0.07	0.018	0.307	0.06
II	5.13	73.17	0.012	0.295	0.04	0.014	0.061	0.23	0.026	0.356	0.07
III	15.90	79.94	0.012	0.387	0.03	0.011	0.007	1.56	0.023	0.394	0.06
IV	31.69	83.92	0.013	0.293	0.04	0.021	0.026	0.79	0.033	0.319	0.10
PEACHES											
I	0.0	89.19	0.022	0.316	0.07	0.081	0.343	0.24	0.103	0.659	0.16
II	1.96	89.22	0.026	0.402	0.06	0.085	0.260	0.33	0.111	0.662	0.17
III	4.11	89.41	0.033	0.561	0.06	0.066	0.174	0.38	0.099	0.736	0.13
IV	6.02	90.00	0.040	0.560	0.07	0.043	0.130	0.33	0.083	0.690	0.12
STRAWBERRIES											
I	—	90.34	0.025	0.228	0.11	0.045	0.388	0.12	0.070	0.615	0.11
II	-	91.13	0.031	0.319	0.10	0.020	0.160	0.13	0.051	0.478	0.11
III	0.0	93.05	0.040	0.319	0.12	0.018	0.081	0.22	0.058	0.400	0.14
IV	14.10	91.58	0.047	0.327	0.14	0.010	0.056	0.18	0.057	0.383	0.15
DEWBERRIES											
I	.	79.93	0.016	0.149	0.11	0.108	0.860	0.12	0.124	1.009	0.12
II	.	84.04	0.021	0.506	0.04	0.053	0.195	0.27	0.075	0.701	0.11

boiling with dilute acid during hydrolysis of the protopectin. Certain experiments (unpublished data) have shown that 17 per cent. or more of the pectin may be destroyed during this period. Were such a correction applied, it would lower the ratios considerably, a number of them to approximately the same value as those from the soluble fraction.

Another factor which would no doubt affect the ratios is the variable amount of polysaccharide. In actual experiment using steam distillation it was found that pure arabinose gave 72 to 84 per cent. of the theoretical yield of furfural, depending principally on the amount of arabinose used. Since the polysaccharide must contain pentose this variable is most likely present.

Leaving the ratios of furfural to pectic substances, we may approach the problem from another angle. If the formation of pectin from protopectin is truly a hydrolytic process, and if the products do not undergo further decomposition, then the sum of pectin and protopectin all calculated as calcium pectate should be a constant for all stages of ripeness. In a like manner, the sums of furfural yields from the soluble and protopectin fraction should be the same for each stage of ripeness. Of course, this would only be apparent providing changes in the weight of the fruit during storage were ascertained and corrected for. Since this was done for bananas and peaches, it is possible in these cases to obtain a check upon this relation. Attention has already been called to the fact that these totals are not entirely constant. While for the pectic constituents there is less variation in peaches than in bananas, nevertheless, in both there is a progressive increase through the first three lots, followed by a decrease in the fourth. While the total furfural increases progressively in bananas it first increases and then decreases in peaches. A correction for loss of pectin during hydrolysis of protopectin nearly eliminates the fluctuation in the pectin totals for peaches but only partially for bananas. It may be that artificial hydrolysis of banana protopectin results in greater destruction of pectin than was found in other tissues. Of course these corrections would not account for the progressive changes in the total furfural. It is thus evident that during ripening, either metabolic changes or decompositions occur which obscure a mathematical verification, or the reaction is not strictly hydrolytic.

Summary and conclusions

It has previously been shown, and is here again substantiated, that during the ripening of fruits pectin increases at the expense of protopectin. It is now shown that concurrently with the development of soluble pectin there is liberated an unidentified furfural-yielding substance, soluble in 70 per cent. alcohol, which increases progressively with the pectin. It is also shown

that when protopectin is decomposed by boiling with dilute acid, more of the furfural-yielding substance is liberated. It is found in decreasing amounts as the amount of protopectin in the fruit is decreased during ripening. Although the ratios of furfural to the different pectic fractions are not entirely constant, the results seem to indicate that this furfural-yielding substance is a component part of protopectin, and is liberated by the splitting of protopectin during the natural ripening process. There is some evidence that certain metabolic changes take place in the pectic constituents themselves, during this period.

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LITERATURE CITED

1. ABBOTT, O. D. The utilization of pentoses by yeasts and the composition of plant gums. Missouri Agr. Exp. Sta. Research Bull. 85. 1926.

2. APPLEMAN, C. O. and CONRAD, C. M. Pectic constituents of peaches and their relation to softening of the fruit. Maryland Agr. Exp. Sta. Bull. 283. 1926.

3. ——————————————. The pectic constituents of tomatoes and their relation to the canned product. Maryland Agr. Exp. Sta. Bull. 291. 1927.

4. CARRÉ, M. H. An investigation of the changes which occur in the pectic constituents of stored fruit. Biochem. Jour. 16: 704–712. 1922.

5. —————— and HAYNES, D. The estimation of pectin as calcium pectate and the application of this method to the determination of the soluble pectin of apples. Biochem. Jour. 16: 60–69. 1922.

6. Committee on Nomenclature of Pectin. DORE, W. H., Chairman. Definitions written by the committee on nomenclature of pectin of the agricultural food division. Proc. Amer. Chem. Soc. 1927. Jour. Amer. Chem. Soc. 49: 37–39. 1927.

7. CUNNINGHAM, M. and DORÉE, C. The production of w-hydroxy-s-methylfurfuraldehyde from carbohydrates and its influence on the estimation of pentosans and methylpentosans. Biochem. Jour. 8: 438–447. 1914.

8. DORE, W. H. The composition of pectin: A preliminary report on the determination of galacturonic acid in pectin. Jour. Amer. Chem. Soc. 48: 232–236. 1926.

9. EHRLICH, F. Die Pektinstoffe, ihre Konstitution und Bedeutung. Chem. Ztg. 41: 197–200. 1917.

10. ——————, and SCHUBERT, F. Über Tetra-araban und seine Beziehung zur Tetragalakturonsäure, dem Hauptkomplex der Pektinstoffe. Biochem. Zeitschr. **203**: 343–350. 1928.

11. —————— and SOMMERFELD, R. V. Die Zusammensetzung der Pektinstoffe der Zuckerrübe. Biochem. Zeitschr. **168**: 263–323. 1926. Chem. Abstr. **19**: 3169. 1925; **20**: 2519. 1926.

12. EMMETT, A. M. An investigation of the changes which take place in the chemical composition of pears stored at different temperatures, with special reference to the pectic changes. Ann. Bot. **43**: 269–308. 1929.

13. FLEURY, P. and POIROT, G. Nouvelle methode de dosage de petites quantites de furfurol par colorimetrie. Jour. Pharm. Chim. **26**: 87–96; Bull. Soc. Chim. Biol. **4**: 251–266. 1922.

14. JOLLES, A. Über ein neues Verfahren zur quantitativen Bestimmung der Pentosen. Zeitschr. anal. Chem. **45**: 196–204. 1906.

15. McCRACKAN, R. F., PASSAMANECK, E., and HARMAN, K. E. Simplified calculations in colorimetry. Jour. Chem. Educ. **3**: 416–418. 1926.

16. McKINNIS, R. B. An investigation of the hypothetical combined pentose and the so-called free pentose with inferences on the composition of pectin. Jour. Amer. Chem. Soc. **50**: 1911–1915: 1928.

17. NANJI, D. R., PATON, D. J., and LING, A. R. Decarboxylation of polysaccharide acids: Its application to the establishment of the constitution of pectins and to their determination. Jour. Soc. Chem. Ind. **44**: 253 T–258 T. 1925. Chem. Abstr. **19**: 2327. 1925.

18. PERVIER, N. C., and GORTNER, R. A. The estimation of pentoses and pentosans. I. The formation and distillation of furfural. Jour. Ind. **44**: 253 T–258 T. 1925. Chem. Abstr. **19**: 2327. 1925.

19. SCHAFFER, F. Ueber die colorimetrische Bestimmung der Pentosen und Methylpentosen im Wein. Mitt. Lebensm. Hyg. **6**: 163–167. 1915.

20. SMOLENSKI, K. Badania nad zwiazkami pektynowemi. Roczniki Chemji **3**: 86–152. 1923. Chem. Abstr. **19**: 41–42. 1925.

21. WICHMANN, H. J. Report on determination of pectin in fruit and fruit products. Jour. Assoc. Off. Agr. Chem. **7**: 107–112. 1923.

22. YOUNGBURG, G. E., and PUCHER, G. W. Studies on pentose metabolism. A colorimetric method for the estimation of furfural. Jour. Biol. Chem. **61**: 741–746. 1924.

EXPERIMENTS WITH *TRIANEA* ON ANTAGONISM AND ABSORPTION

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These experiments were undertaken in order to check by a different technique observations previously reported (27) in which seedlings of *Phaseolus sp.* and *Lupinus albus* were used. The following phenomena were observed: (1) that small concentrations of the Fe ion increased the growth rate of seedlings; (2) that the presence of the Ca ion inhibited, if it did not eliminate, the killing effect of certain otherwise lethal concentrations of Fe; and (3) that relatively high temperature (approximately 29° C.) increased the degree of permeability of the roots even though great amounts of Ca were present. The data to be presented here confirm the foregoing observations and present evidence indicating that Ca decreases the degree of permeability of cells to a marked extent.

General Considerations

All other plant requirements being normal, it is the opinion of the writer that the distribution of plants is a function of the Ca supply and of toxic substances, for the most part Fe and Al. That is to say, given a soil that can support a vegetation, the type that dominates or survives will be determined by the Ca, Fe and Al content of the soil colloids or soil solution. The pH of the soil or soil extract is believed to be a negligible factor (26) although it is a significant criterion of the concentration of ions to be expected on analysis of soil water (1, 6, 13). From a turning point at pH 5.0, Fe and Al are increasingly present in the soil extract, the concentration varying with the phosphate content as well as the pH of the soil solution (1, 6, 13, 14, 25). That some plants cannot survive a greater concentration than approximately 1.0 ppm. of Al or Fe has been shown in a recent paper (27).

It remains to consider why some plants require a relatively high Ca content in the growth medium while others are indifferent to the concentration of this ion; why some plants are so sensitive to the presence of Al and Fe, and why others are comparatively resistant. There are many observations bearing on these points.

Nägeli (16) observed that when the calciphile *Achillea atrata* and the calciphobe *A. moschata* occur in the same valley, each is confined to its own soil type; but if either occurs in the absence of the other, it is non-discriminating. It is the opinion of Schimper (24, see 22 and 23 for extensive references) that the failure of plants on chalk is due to the difficulty in

absorbing sufficient Fe, and that such failures may be obviated by watering with an iron solution. KERNER (9) has compiled a long list of contrasting plant species. Among them are *Rhododendron hirsutum* and *R. ferrugineum, Androsace pubescens* and *A. glacialis, Juncus hostii* and *J. trifidus.* The former of each contrasted plant pair is the calciphile, which, in order to survive, must have a soil which is relatively high in Ca. The latter of each contrasted plant pair is the calciphobe. Such plants either grow very slowly in a calcium rich soil or are indifferent to the presence of Ca. PAUL (19), in working with various *Sphagnum* species, found that those indigenous to high moors, where the salt supply is low, are normally much less resistant to Ca than those growing on the low moors where the minerals are more abundant. Less than 90 ppm. of lime were sufficient to cause death to the high moor *Sphagnum,* while over 200 ppm. were needed to kill the low moor species. The observations of SKENE (28) are of interest on this point—*Castanea* can grow on a chalky soil when supplied with abnormally large amounts of K, indicating that the chalk acts by interfering with the supply of other salts through the roots. He is of the opinion that "mineral solutions are generally physiologically harmless, but may be ecologically harmful." LOEB (11) records that $CaCl_2$ inhibits the twitching of muscles or nerves caused by Na_2SO_4, sodium citrate, or $N(C_2H_5)_4Cl$, which may be due to the prevention of diffusion through the membrane or to the action of Ca on protoplasm. Evidence points to the former view. In a later paper (10), he states that the diffusion of KCl through a membrane is due to a change in the external part of the membrane produced by the presence of other salts. Finally in a recent paper by HARDENBURG (5) the statement is made that "the increased applications of lime to lettuce reduced the growth of both tops and roots, decreased the total ash content and the absorption of Al, Fe, and Ca to a moderate extent."

Other citations are at hand but there is little to be gained by repetition. A great body of observation and data indicates most strongly that Ca decreases the degree of permeability of the plasma membrane with respect to substances entering the cell. Some plants find this essential (calciphiles) while others starve slowly or rapidly under the same conditions (calciphobes). This paper attempts to demonstrate the truth of LOEB's observation "that the external part of the membrane only is affected."

The various degrees of sensitivity of plants to Fe and Al seem to be coincident with their comparative indifference to Ca. This is largely conjectural as there are few data on the subject. From the preceding statements, the proof would necessitate the demonstration of variability in the plasma membranes (or, more conservatively, the protoplasm) of the plants considered. HANSTEEN-CRANNER's work (4) is most suggestive. He con-

cludes that there are water-soluble and water-insoluble lipoids making up the plasma membrane. Should this be the case, it would follow that those plants having small quantities of water-insoluble lipoids would require large amounts of Ca to maintain a water-in-oil emulsion at the free surface. A possible mechanism is explained by Clowes (3). Contributions on the temperature relationships to the viscosity of protoplasm and to the permeability of the cell agree with this opinion (4, 7, 12, 18, 27).

The writer inclines toward this view but suggests a second, simultaneously operative. Even though the plasma membranes of root hairs and epidermal parenchyma cells are permeable, however slowly, to Fe and Al (as well as other cations, both toxic and relatively harmless), the cells of certain plants are not affected. This apparent indifference may be due to the precipitation within the cell of the Fe and Al ions as insoluble complex salts or esters, by ions already present within the cell and harmless to the cell in the quantities present. Two such kinds of compounds are probable, tannins and oxalates. The presence of the former in cells in significant quantities has been known for decades. Molisch (15) has ascertained the presence or absence of oxalates in 246 plant species.

This paper shows that the presence of tannin, sodium oxalate and haematoxylin in the root hairs of *Trianea* eliminates the toxic action of Fe for a considerable period even though Fe enters the cell constantly.

Methods

Immersion

The root hairs of *Trianea bogatensis* were the material used. Under ideal conditions, the protoplasm of the root hairs streams for hours when the roots are immersed in distilled water. The rate is constant for any reasonable time interval taken. A root from a healthy plant was cut and immersed and irrigated with distilled water. The cyclotic rate was measured by means of a stop watch and a graduated micrometer eyepiece. The protoplasm is normally very mobile and liquid in appearance and its viscosity appears to be low. There are particles present which either are suspended in the protoplasmic streams or which make up the living material itself. These are easily designated and observed and they vary in size from about 2–6 micra in diameter. Sometimes, indeed, they are much larger, in which case they constitute a specific gel. The rate of movement is independent of size.

Many streams of protoplasm can be observed in simultaneous motion. Generally one main stream moves from the tip to the base of the hair while a stream on either side of the hair progresses in the opposite direction.

From time to time, new currents appear at the expense of those already present. It is most convenient to measure the time necessary for a given particle to travel 50 micra. From 8–12 careful measurements can be made in a five-minute interval. When including all moving streams, this gives a fair cyclotic average. The results obtained for any five-minute interval constitute the average speed for that interval and are so reported.

Determinations on the rate of cyclosis of the protoplasm of a hair in distilled water have been made over a period of three hours. For any given five-minute interval, the cyclotic rate has varied less than 10 per cent. in carefully grown and selected material. This being the case, it was considered important to take only sufficient determinations to obtain a reasonable estimate of the cyclotic rate of the chosen hair in distilled water. This rate is the basis of comparison for subsequent experiments using the same hair. The time interval chosen was 20 minutes. Many such experiments were made simply as checks after an experiment of long duration was completed.

After the rate of cyclosis of a given hair had been determined in distilled water, the same hair was irrigated with a solution of 10 ppm. of Fe (as the nitrate) and 20 ppm. of Ca (as the chloride). Hairs irrigated with this solution have been observed for two hours on several occasions. The rate of cyclosis does not vary significantly and remains practically the same as that recorded for distilled water. Therefore, it was thought necessary in check experiments to make observations only over a time interval permitting a reasonable estimate of the rate of streaming. For check experiments, 20 minutes again was chosen.

The same root was then irrigated with a solution of ferric nitrate containing Fe at the rate of 10 ppm. The rate of cyclosis was determined for five-minute intervals until the death of the observed hair.

<center>INJECTION</center>

The micro-manipulation method and micro-injection have been described by CHAMBERS (2) and PÉTERFI (20). The CHAMBERS micro-dissection apparatus was used in this work. With micro-pipettes with external measurements of 3 or 4 micra, water, calcium chloride (20 ppm. of Ca), ferric nitrate, haematoxylin, tannin, and sodium oxalate were injected into the base of the root hairs.

Only pipettes which penetrate the cell wall with the greatest ease should be used. Otherwise mechanical injury to the hair becomes a most important factor. A good injection, so far as the writer can determine, offers no significant mechanical injury as judged by the rate of streaming of the protoplasm and its general appearance. This point was determined in the

following way: Five different hairs were pierced but not injected at intervals of five minutes. The rate of streaming was determined for each hair before it was pierced, then for five minutes after piercing and finally a half hour after piercing. All continued normal over the intervals mentioned. In one hair, however, cyclosis ceased for two minutes after puncturing; in three hairs for 30 seconds; and in the fifth hair no period of suppression was observed. Distilled water has no lasting effect on the cyclotic rate or on the health of the cells when it is injected.

It is believed, therefore, that any results observed after injecting a substance into the hairs are due to the reaction of that substance on the cytoplasm of the hairs and to nothing else.

The solutions of the salts of Fe, Ca, and Ca + Fe were injected into two or more hairs on a single root and the root then irrigated with distilled water while the observations were made. The numerous uninjected hairs on the same root served as a very excellent multiple check to observed reactions.

When solutions of haematoxylin, tannin, and sodium oxalate were injected while the root was suspended in a hanging drop of distilled water, the root was put immediately on a slide and irrigated with a solution of ferric nitrate (10 ppm. of Fe). Observations were made using uninjected hairs as checks.

In all cases the base of the hair was punctured. The opening quickly healed with a gel of protoplasm forming a plug which almost immediately became a brown coagulum. Other effects are described separately.

CRITICISM OF METHODS

The most serious objection to the material employed is the difficulty in reproducing the results recorded. The root hairs of *Trianea* are exceedingly delicate cells and their "degree of health" varies greatly. This work was begun in December, 1927, and in the following five months the immersion experiments were concluded. At the time, it was observed that temperature played a most important rôle in toxicity. In order to obtain a curve, the experiments were continued. Unfortunately, it was found impossible to reproduce results with the root hairs then obtainable. New stolons were rooted and experiments repeated with the new crop the following autumn, without success. In spite of many efforts, suitable material was not obtained until the spring of 1929, but it was found impossible to conclude the work before the advent of hot weather. In order to complete the task, it became necessary to grow the plants in the following manner. Healthy plants were rooted in heavy garden soil in deep glass containers. The containers were immersed in 6-gallon jars and the new

stolons permitted to project into and root in the surrounding water. The 6-gallon jars were put into tubs filled with water cooled to 16° C. ± 3°. This kept the surface inch of water occupied by the roots of the daughter plants at a temperature of 18° C. ± 2°. In three weeks, sufficient roots were available to continue work. The hairs thus obtained were resistant to the treatment described and with them the entire work was repeated. Consistent reproducible results were obtained. Similar stock grown simultaneously in tanks with the temperature at 24° C. ± 3° were entirely unsuited for immersion experiments because of the great sensitivity of the hairs.

Experiments, therefore, are here considered as individual cases demonstrating a consistent tendency but not necessarily the degree of that tendency.

With respect to the micro-injection experiments, little effort was made to be quantitative. It is impossible as yet even to estimate the quantity of liquid injected. One can always be certain of an injection by the observable, though slight, displacement of particles in or of the protoplasm.

In the immersion experiments, only the most resistant roots were employed, while in injection, because of the time factor, very sensitive plants were chosen.

Results

IMMERSION

Table I shows the results of two experiments in which root hairs were irrigated first with distilled water, and then with ferric nitrate in the concentration of 10 ppm. of Fe.

It is apparent that this concentration of Fe causes a most pronounced acceleration in the rate of cyclosis soon after it is added as compared with the rate recorded for distilled water. The rate of streaming gradually decreases and finally stops. The protoplasm gradually aggregates at the tip of the hair and, just before death, moves with pulsating jerks and quickly turns a decidedly brown color. The tip of the hair may or may not burst. Should death occur quickly, the tip perceptibly swells and the protoplasm is extruded with a peculiar pumping motion.

Table II presents data of two similar experiments. The roots were irrigated with distilled water for the time interval indicated, then with a solution containing 10 ppm. of Fe and 20 ppm. of Ca, and finally with a third solution containing only 10 ppm. of Fe. These experiments are typical of a large number of similar observations.

In the presence of Ca it is noted that Fe has no effect on the cyclotic rate within the limit of the experiments. As previously remarked, no change was observed when this part of the experiment was twice continued for two hours. As soon as Ca is removed from the irrigating liquid, the

TABLE I

CYCLOSIS OF *Trianea* ROOT HAIRS IMMERSED IN WATER, FOLLOWED BY FERRIC NITRATE

IRRIGATING LIQUID	INTERVAL OF OBSERVATION	CYCLOSIS, PER SECOND
	minutes	μ
Water	5	4.2
Water	10	4.1
Water	15	4.2
Fe, 10 ppm.	5	4.8
Fe, "	10	5.7
Fe, "	20	4.9
Fe, "	25	3.6
Fe, "	35	3.9
Fe, "	50	3.6
Fe, "	90	3.0
Fe, "	100	No movement, brown, granular coagulum, death
Water	5	4.5
Water	10	4.2
Water	15	4.7
Water	20	4.4
Fe, 10 ppm.	5	4.5
Fe, "	10	5.5
Fe, "	15	5.3
Fe, "	25	7.3
Fe, "	35	5.0
Fe, "	40	5.1
Fe, "	50	3.9
Fe, "	55	Broke at tip, no movement, brown, granular coagulum, death

toxic action of Fe is exhibited in the increased rate of streaming, the following decrease, and characteristic death.

It is concluded that Ca antagonizes Fe in the concentrations used and that this antagonism is effected at the surface although not necessarily confined to the surface.

INJECTION

CA 20 PPM. (AS THE CHLORIDE).—When Ca is injected into the hair, a gel quickly appears in the region of injection and ·slowly becomes a coagulum. The rate of streaming decreases sharply until there is practically no movement of the protoplasm within 5 minutes. The gel is produced progressively from the point of injection at the base of the hair toward the tip, and death by the formation of a coagulum ensues in 10–15 minutes. Cells have been observed to produce a gel in about half the protoplasmic contents and then to convert it slowly into a sol, the entire hair becoming normal within 59 minutes after injection. Evidently this is a

TABLE II

ANTAGONISM OF CA AND FE ON CYCLOSIS OF *Trianea* ROOT HAIRS

IRRIGATING LIQUID	INTERVAL OF OBSERVATION	CYCLOSIS, PER SECOND
	minutes	μ
Water	5	5.5
Water	10	5.0
Water	15	5.3
Water	20	5.5
Ca, 20 ppm. + Fe, 10 ppm.	5	5.2
Ca, " + Fe, "	15	5.0
Ca, " + Fe, "	40	5.5
Fe, 10 ppm.	5	5.5
Fe, "	10	7.0
Fe, "	20	7.9
Fe, "	40	4.3
Fe, "	80	
Fe, "	85	Barely perceptible, no movement; brown, granular coagulum, death
Water	5	6.6
Water	10	6.0
Water	20	6.3
Ca, 20 ppm. + Fe, 10 ppm.	5	6.9
Ca, " + Fe, "	10	6.6
Ca, " + Fe, "	20	6.5
Ca, " + Fe, "	25	6.1
Fe, 10 ppm.	5	8.5
Fe, "	10	6.6
Fe, "	15	3.0
Fe, "	20	2.8
Fe, "	25	No movement; brown, granular coagulum; death

function of the quantity of Ca injected. Ca shows a decided toxic action on the hairs when injected, whereas no effect is observable when the hairs are immersed in much greater concentrations of the same ion (0.001 M.).

When compared with the reaction of the hairs on immersion in calcium chloride, the experiment implies that Ca is very slow in penetrating the cell; in fact, so slow as to be considered physiologically non-penetrating with respect to the criteria here used.

FE 10 PPM. (AS THE NITRATE).—Two types of results have been obtained with the injection of Fe, which the writer believes are simply functions of

the quantity of Fe injected into the cells. After injection the cyclotic rate is often increased similarly to the tendency reported in tables I and II for the immersion experiments. In other cases, the rate of protoplasmic streaming immediately decreases and the coagulation of the protoplasmic contents and the death of the cell occurs much faster than in the former instance. The sequence of events in the two types of reaction follows:

1. After injection, there is apparent a pronounced acceleration in streaming, beginning from 3–6 minutes after injection and continuing from 2–4 minutes. The rate then rapidly decreases, becoming almost imperceptible within 15 minutes. In upwards of 30 minutes the gel appears, which quickly becomes a coagulum. This coagulum is produced simultaneously in all parts of the hair. The protoplasm is tinted brown, and the hair is dead.

2. After injection, the streaming continues normal for perhaps 2 minutes, at which time the cyclotic rate decreases rapidly, becoming almost imperceptible within 10 minutes. A coagulum is formed simultaneously in all parts of the cell in from 15–20 minutes after injection, which rapidly becomes brown, and the cell is dead.

The toxicity of Fe when injected is patent. It causes a marked granular appearance of the protoplasm after death. The acceleration of the protoplasmic streams has been observed many times but the second method of death above described is more usual.

CA 20 PPM. (AS THE CHLORIDE) + FE, 10 PPM. (AS THE NITRATE).—In no experiment was the rate of streaming observed to increase when this mixture was injected. On the contrary, the reaction of the protoplasm was apparently the same as though $CaCl_2$ alone were injected. However, the rate of cyclosis rapidly decreases and a gel is produced at the point of injection. This gel progresses toward the tip of the hair and progressively becomes a coagulum. Death occurs in from 10–15 minutes.

INJECTION AND IMMERSION

SODIUM OXALATE (SATURATED SOLUTION).—Root hairs were injected with this solution and then irrigated with ferric nitrate (10 ppm. of Fe). Table III shows the results of a typical experiment.

Immediately preceding the formation of the coagulum, a marked vacuolization occurs with intermittent streaming. On death, the granular structure produced is much less vacuolar.

From the cited experiments, it is observed that the injected hairs survive immersion in a lethal Fe solution averaging 240 per cent. longer than the uninjected hairs. The most favorable experiment at hand using sodium oxalate shows that the average life of 3 injected hairs is 350 per cent. longer than that of the uninjected hairs when immersed in a toxic Fe solution.

TABLE III

RESPONSES OF ROOT HAIRS OF *Trianea* TO INJECTION OF SODIUM OXALATE FOLLOWED BY
IMMERSION IN FERRIC NITRATE

TIME IN MINUTES	REMARKS
0	Injected 4 hairs, observed 2
5	Immersed root in Fe solution (10 ppm. of Fe)
7	Many uninjected hairs burst at tip
10	All uninjected hairs burst at tip
	Injected hairs normal
13	Injected hairs reducing cyclotic rate; protoplasm in one hair very viscous
15	1 injected hair has formed a coagulum, brown, granular, dead
19	Second injected hair has formed coagulum, brown, granular, dead

TANNIN (FIFTH SATURATED SOLUTION).—Injected with this solution, root hairs were immediately irrigated with a solution of ferric nitrate (10 ppm. of Fe). The results are presented in table IV.

TABLE IV

RESPONSES OF ROOT HAIRS OF *Trianea* INJECTED WITH TANNIN SOLUTION, FOLLOWED BY
IRRIGATION WITH FERRIC NITRATE

TIME IN MINUTES	REMARKS
0	3 hairs injected, 2 observed
1	Immersed in ferric nitrate (10 ppm. of Fe)
3	Many cells burst
4	All uninjected cells burst, injected cells normal
14	Rate of cyclosis decreasing, protoplasm massing at tip, hair becoming vacuolate
17	Streaming almost imperceptible
20	Coagulum formed in 1 cell, brown, granular; vacuoles disappear to some extent
22	Coagulum formed in second cell, brown, granular; vacuoles smaller and fewer

Tannin is somewhat toxic to these cells when injected. A saturated tannin solution causes the coagulation and death of the cells within a few seconds after injection. Half saturated tannin solutions were also unsatisfactory. However, a fifth saturated tannin solution is apparently harmless to the cells in the quantities injected within the time limits of the experiments.

It is remarked that cells injected with tannin live 650 per cent. longer in a lethal iron solution than uninjected cells. This duration is average for the experiments performed.

HAEMATOXYLIN (SATURATED SOLUTION).—Injected with this solution, root hairs were irrigated with a solution of ferric nitrate (10 ppm. of Fe). The tendency is indicated in table V.

TABLE V

RESPONSES OF ROOT HAIRS OF *Trianea,* INJECTED WITH SATURATED HAEMATOXYLIN, AND IRRIGATED WITH FERRIC NITRATE

TIME IN MINUTES	REMARKS
0	Injected 6 hairs, observed 2
2	Immersed in ferric nitrate (10 ppm. of Fe)
4	All uninjected hairs burst
6	Slight acceleration in streaming in injected hairs
12	Cyclosis normal
15	Decrease in rate of streaming
18	Streaming perceptibly decreases
19	Formation of vacuoles, no cyclosis
20	Coagulum formed in one hair, brown, granular; dead. Decrease in number of vacuoles
24	Cyclosis imperceptible in second hair
26	Brown, granular coagulum formed, dead. Few vacuoles present

In a lethal iron solution, hairs injected with haematoxylin live 1100 per cent. longer than the uninjected hairs.

It is apparent that the injection of haematoxylin, tannin and sodium oxalate into the root hairs of *Trianea* enables those root hairs to survive immersion in toxic and lethal iron solutions significantly longer than uninjected root hairs. Depending on the quantity of injected material in the cells, the time advantage of survival is indefinitely increased.

Discussion

1. The relative non-penetration of Ca.

Small quantities of Fe injected into the cells show a great toxic action. Immersion experiments with this toxic ion exhibit practically the same results with the same material. Immersion experiments with Ca in concentrations up to 0.001 M. show no effect whatever on the protoplasm of the hairs but when small dilute quantities are injected, death results through the formation of a gel, followed by irreversible coagulation. If Ca were as penetrating as Fe, the same results, or at least comparable results, should be obtained on immersion as well as on injection.

2. The antagonism of Ca for Fe.

Immersion experiments (table II) clearly indicate this antagonism. Fe does not enter the cell because Ca is present in the same solution. This action of Ca is strictly a temporary effect for as soon as it is removed, the

characteristic Fe toxicity appears. The root hairs behave precisely the same whether they are *immersed* in an Fe solution or *injected* with same Fe solution.

3. "The external part of the membrane only is affected."

From the above data, this conclusion must follow. Injections are made into the vacuole of the cells. Indeed, it would be almost impossible to inject the cytoplasm. When Fe alone is injected it diffuses rapidly to all parts of the hair, causing simultaneous death of all the protoplasm. Ca remains rather local in its effects, although toxic, forming a viscous gel. The gel with Ca has never been observed in any of our immersion experiments. Therefore no significant quantities of Ca penetrate the cytoplasm beyond the immediate periphery (the plasma membrane) when root hairs are immersed in solutions containing this ion. When the hairs are immersed in solution of both Ca and Fe ions, cyclosis remains normal as does the general appearance of the protoplast. Did Fe penetrate beyond the immediate surface, the effect of its entrance should parallel that seen in injection experiments. Therefore, Ca antagonizes Fe by decreasing the degree of permeability of a definite physiologic, peripheral membrane, the plasma membrane. These observations are in harmony with a great deal of other work (7, 8, 17, 21).

These data indicate (but do not demonstrate) that the plasma membrane is composed of a different type of cytoplasm than the vacuolar membrane. Otherwise Ca should remain isolated in the vacuole when injected there and should antagonize Fe by its action on the vacuolar membrane. That this is not the case seems likely.

4. The protective action of substances dissolved in the vacuole.

When cells of great "vitality" or "degree of health" were injected with solutions of tannin, oxalate, or haematoxylin and immersed in toxic Fe solutions, they survived the uninjected cells for hours. Such experiments were impractical and so the time advantage of "sensitive" hairs is recorded. These substances in the concentrations and amounts used are not harmful to the cells. Whether they remain in the vacuole or diffuse out of the cells is conjectural. But so long as they are present, toxic Fe solutions have no effect, although the Fe enters the cell. This latter statement is checked in two ways:—First, the check cells are quickly killed; and second, when the injected substances are precipitated, the injected cells are killed. It is believed, but not demonstrated, that such protecting substances are an ecologic factor in the distribution of plants. The work of MOLISCH (15) is suggestive on this point.

5. The effect of temperature on the rate of permeability.

When *Trianea* is grown below 14° C. for over a week, the cyclotic rate is consistently slower than when the plants are kept at higher temperature. Also the hairs appear to be less permeable to toxic Fe solutions. Material

grown above 23° C. is extremely permeable to the same Fe solutions; and, further, Ca appears powerless to decrease the degree of permeability of the cells.

Conclusions

1. In single salt solution, Fe readily penetrates the root hairs of *Trianea bogatensis*.
2. Ca is a relatively non-penetrating ion.
3. Ca decreases the rate of entry of Fe into the hairs.
4. In the antagonism of Ca for Fe, the "external part of the plasma membrane only is affected."
5. Some evidence is presented suggesting that the plasma membrane is physiologically different from cytoplasm.
6. Such substances as sodium oxalate, tannin, and haematoxylin, when present in the cell, protect the cell from the lethal action of Fe.

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LITERATURE CITED

1. BURGESS, P. S. Comparison of "active" aluminum and hydrogen-ion concentrations of widely separated acid soils. Soil Sci. **15**: 407–412. 1923.
2. CHAMBERS, R. The physical structure of protoplasm as determined by micro-dissection and injection. General Cytology, 237–309. Chicago. 1924.
3. CLOWES, G. H. A. Protoplasmic equilibrium. I. Action of antagonistic electrolytes on emulsions, and living cells. Jour. Phys. Chem. **20**: 407–451. 1916.
4. HANSTEEN-CRANNER, B. Beiträge zur Biochemie und Physiologie der Zellwand und der plasmatischen Grenzschichten. Ber. deutsch. bot. Ges. **37**: 380–391. 1919.
5. HARDENBURG, E. V. Muck-soil reaction as related to the growth of certain leaf vegetables. Plant Physiol. **3**: 199–210. 1928.
6. HARTWELL, B. L., and PEMBER, F. R. The presence of aluminum as a reason for the difference in the effect of so-called acid soil on barley and rye. Soil Sci. **6**: 259–279. 1918.
7. HEILBRUNN, L. V. The colloid chemistry of protoplasm. 3. The viscosity of protoplasm at various temperatures. Amer. Jour. Physiol. **68**: 645–648. 1924.
8. —————————. The physical structure of the protoplasm of sea-urchin eggs. Amer. Nat. **60**: 143–156. 1926.
9. KERNER, A. Ueber das sporadische Vorkommen sogenannter Schieferpflanzen im Kalkgebiege. Verhd. Zoolog-botan. Ges. in Wien. **13**: 245–256. 1863.

10. Loeb, J., and Ewald, W. F. Chemical stimulation of nerves. Jour. Biol. Chem. **25**: 377–390. 1916.
11. Loeb, J. The mechanism of the diffusion of electrolytes through the membranes of living cells. I. The necessity of a general salt effect upon the membrane as a prerequisite for this diffusion. Jour. Biol. Chem. **27**: 339–352. 1916.
12. McCutcheon, M., and Lucke, B. The kinetics of osmotic swelling in living cells. Jour. Gen. Physiol. **9**: 697–707. 1925–1926.
13. Magistad, O. C. The aluminum content of the soil solution and its relation to soil reaction and plant growth. Soil Sci. **20**: 181–225. 1925.
14. Mirasol, J. J. Aluminum as a factor in soil acidity. Soil Sci. **10**: 153–217. 1920.
15. Molisch, H. Über den mikrochemischen Nachweis und die Verbreitung gelöster Oxalate im Pflanzenreiche. Flora 111–112: 60–70. 1918.
16. Nägeli, C. Von. Ueber die Bedingungen des Vorkommens von Arten und Varietäten innerhalb ihres Verbreitungsbezirkes. Sitzungsber. bayr. Akad. **2**: 367–395. 1865.
17. Osterhout, W. J. V. On the decrease in permeability due to certain bivalent cations. Bot. Gaz. **59**: 317–330. 1915.
18. ————. Does the temperature coefficient of permeability indicate that it is chemical in nature? Bot. Gaz. **63**: 317–330. 1917.
19. Paul, H. Zur Kalkfeindlichkeitsfrage der Torfmoose. Ber. deutsch. bot. Ges. **24**: 148–154. 1906.
20. Péterfi, T. Die Mikrurgie der Gewebskulturen. Arch. exp. Zellforsch. **4**: 165–174. 1927.
21. Runnström, J. Eine Lipoide Oberflächenschicht bei dem Seeigelei. Acta Zoolog. **4**: 285–311. 1923.
22. Schimper, A. F. W. Ueber Kalkoxalatbildung in den Laubblättern. Bot. Zeit. **46**: 65–69. 1888.
23. ————. Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze. Flora **73**: 207–261. 1890.
24. ————. Pflanzengeographie. Jena. 1898.
25. Skeen, J. R. Effects of some electrolytes on kaolin and the probable relation to the soil. Soil Sci. **23**:225–242. 1927.
26. ————. Some reactions of seedlings to weak concentrations of hydrochloric acid and calcium. Soil Sci. **26**:471–478. 1928.
27. ————. The tolerance limit of seedlings for aluminum and iron and the antagonism of calcium. Soil Sci. **27**: 69–80. 1929.
28. Skene, M. The acidity of *Sphagnum* and its relation to chalk and mineral salts. Ann. Bot. **29**: 65–87. 1915.

ENERGY EMANATION DURING CELL DIVISION PROCESSES
(M–RAYS)

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(WITH THREE FIGURES)

Experiments with M-Rays (mitogenetic rays) conducted by the author since September 1928 are partly a continuation of work done by a small group of Russian investigators whose only technical mistake perhaps has been somewhat too soon to call themselves "Practical Vitalists." The problem of the M-Rays (mitogenetic rays) has very little to do with vitalism as a doctrine, but its study has presented some new facts pertaining to the field of biophysical phenomena which occur during cell division processes in general.

HABERLANDT's (10, 11, 12) theories of cell-division hormones, wound-hormones, necro-hormones, lepto-hormones, etc., affecting cell division and the growth of tissues as a whole, are a purely biochemical explanation of some cellular processes. The physical side of the problem was not at all taken into consideration by HABERLANDT.[1]

An explanation on a purely biophysical basis has been presented by GURWITSCH (4) in his experiments on the induction of cell division processes in the onion root meristem by exposing it to another onion root. Biophysical induction has been more recently found to occur in plants and protista[2] by RAWIN (16) in sunflower root tips; FRANK and SALKIND (3) in sunflower seedling leaves; KISLIAK-STATKEWITSCH (13) in potato leptom; WAGNER (19) in *Vicia faba* root-tips; BARON (1) in yeast cultures; MAGROU and MAGROU (14) in *Bac. tumefaciens;* BARON (2) in *Bac. anthracoides, Sarcina flava, Bac. coli commune,* etc. Most of this work has been done in Russia, but the results have been published in French and German journals.

Owing to the established prestige of HABERLANDT, the biophysical explanation of phenomena which he regarded as biochemical, has not gained popularity. The results obtained by Russian colleagues have been ignored in Western Europe for about seven years, until first efforts of verification

[1] WENT, (20), mentioned that his son found that there is definite emanation from the upper part of a plant (tip) which affects the lower part (stump) by changing the direction of its growth, the two parts being completely separated by the interposition of an artificial culture medium. The explanation suggested was also of a biochemical nature.

It has been noticed also that two pieces of homologous animal tissue planted together but at a short distance from each other (in tissue culture), stimulate the growth of each other. In this case no definite explanation has as yet been offered.

[2] Animals not mentioned.

of the results obtained by them were made by MAGROU and MAGROU (14) in Lyon, France.   MAGROU repeated the experiments with success and has expressed himself in favor of the presence of inductive forces.   Positive results outside of Russia have been also obtained so far by WAGNER (19) in Prague; REITER and GABOR (17) in Berlin Siemensstadt; and by BORODIN in New York.

ROSSMAN (18) in Rostock repeated the original experiments with the onion-root meristem but failed to obtain positive results.   This may, however, be ascribed to inadequate technique.   His protocols, before publication, were used by his colleague, VON GUTTENBERG (8) of Rostock, in the three articles in which VON GUTTENBERG published a very sharp criticism of the theory of M-Rays (mitogenetic rays).   VON GUTTENBERG himself never did any of the experimental work in this field.

This short report on experiments with M-Rays prepared for the New York meeting of the American Association for the Advancement of Science in 1928 is confined to plant tissues and, therefore, does not include results obtained by me with animal tissues, nor does it include my studies on the physical and optical properties of the M-radiations.

The technique for the detection of M-Rays (mitogenetic rays) from the onion-root meristem has not yet been described in English.   The following is only a brief statement of the technique which will be described in detail in a forthcoming review.

A single onion root connected with its whole bulb, or with a part of its bulb, was placed in a vertical capillary tube of quartz (fig. 1 D) and kept moist.   The meristem of this root was used as the "detector" of the radiation.   Another onion root, also connected with its bulb (entire or in part), was placed horizontally in a glass capillary tube (fig. 1 S) and also kept moist, and used as the "sender" of radiation.   Both tubes are fixed to a special "inductorium," consisting of glass or celluloid plates 7.5 x 5.25 mm. with three more small plates cemented to it in such a way that the distance between the plates is about 1.25 mm. (fig. 1).

In such a position the roots (fixed in the inductorium) were exposed for varying times, from one hour to 2 hours and 30 minutes.   The unexposed side of the "detector" was marked with India ink after the induction, and then immediately fixed in Bouin-Allen fluid, etc., for the purpose of sectioning.

In fixed and stained sections the mitoses on the "induced" (exposed) and "noninduced" (nonexposed) sides were counted and the difference in numbers is given in percentages to show the relation between the number of mitoses on the exposed and nonexposed sides.   This difference given in percentage we shall call the percentage of induction or, in brief, "Ind. per cent."
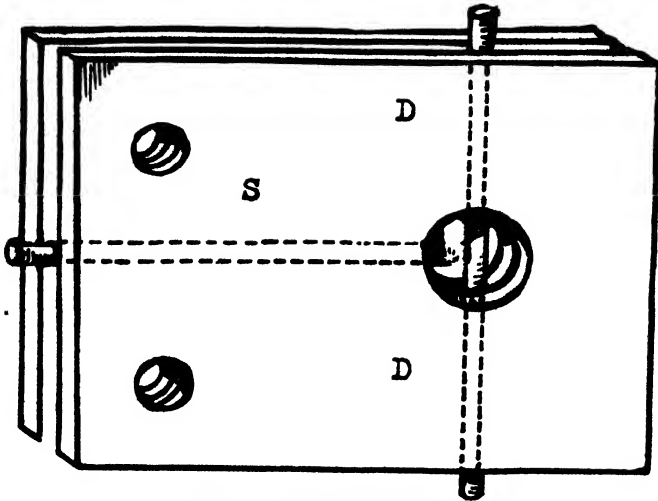
FIG. 1. Inductorium No. 1.

The results of the experiments with onion-root meristem, when the inductoria described above were used, are all positive, as is shown in tables later. It will be seen, however, that the induction percentage varies to a considerable extent. This variation can be explained by the fact that the centering of the "sender" on the "detector" was not fully controlled. In a later experiment the centering was better controlled by using a system of good Leitz mechanical stages together with a horizontal microscope. This equipment was still later replaced by a special inductorium which I have named "condenser." Of this type two modifications were made.

The first modification of inductorium-condenser was made by using an additional capillary glass tube with another onion root as an extra sender. Later, about ten capillary glass tubes (fig. 2) have been used and placed radially on one common glass or celluloid plate of 7.5 x 5.25 mm., pointing them to a common center, which was at a distance of 1 mm. from the end of each tube. In this center the quartz capillary tube with a detector root has been placed vertically, as is shown in fig. 2 D. The results obtained by this type of inductorium were more uniform.

The "condenser" just mentioned has worked quite satisfactorily, but finally was substituted by two object glasses, or celluloid plates, of the same size fastened together. The onion root mash, or other senders, was placed and fixed between these plates as is shown in fig. 3 S. This "sender holder" with some modifications has been quite satisfactory.

In these experiments the onion-root-tip meristem was used, since it is an object which already has been studied very carefully by other investigators.[3]

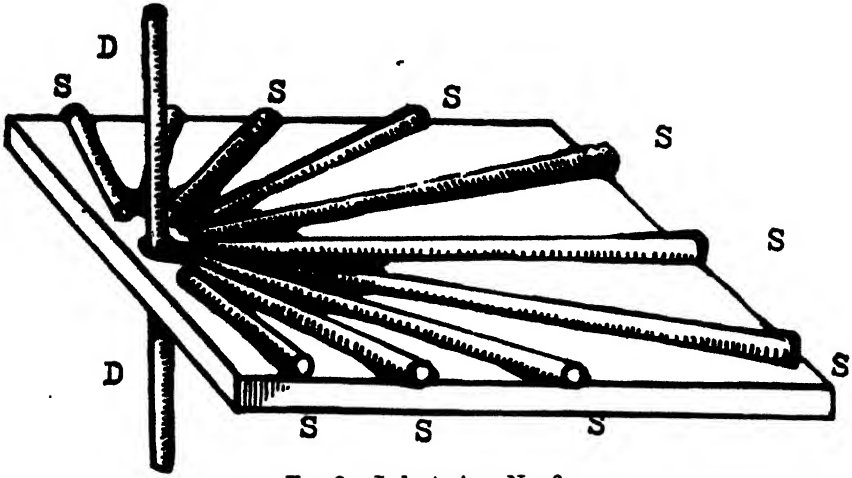3 The average deviation in number of mitoses in opposite sides is estimated as ± 8 – 13 per cent.

Fig. 2. Inductorium No. 2.

The first experiment was with homoinduction. The "sender" in this set of experiments was a fresh, healthy onion root of one of the three commercial varieties common on the markets of New York: Spanish, Texas red, and Texas white. The "detector" was another onion root of the same variety.

I offer two new signs: ≠, to indicate the "sender;" and ⊙ to indicate the detector.

The purpose of this experiment was, first, to prove the existence of energy emanation from the onion-root tip; second, to prove the existence of an induction at a distance and through media which prevent the possibility
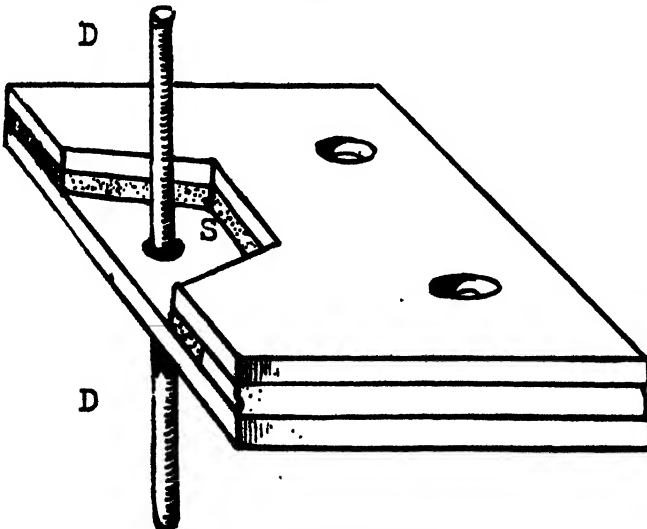
Fig. 3. Inductorium No. 3.

of chemical action; in other words, to prove the physical character of induction.

Homoinduction

The protocols of the experiments with homoinduction are shown below.

EXPERIMENT NO. 11c

Homoinduction: Sender ⇥, onion root tip, variety Texas white. Detector ⊙, same variety, meristem. Apparatus, inductorium no. 1. Separation, quartz capillary tube, and water. Distance, 1 mm. Time of exposure, 2 hours 15 minutes. Fixation, by Flemming. Staining, by iron haematoxylin in sections of 10 μ thickness.

	Mitoses			Resting full-sized nuclei		
(a) Exposed side mitoses, per cent.	31	37	42	41	38	51
(b) Unexposed side mitoses, per cent.	25	30	38	39	27	38
(c) Difference	6	7	4	2	11	13
(d) Induction, per cent.	+24	+23	+11	+5.1	+40	+34

Results: Definite induction of onion-root meristem through quartz.

EXPERIMENT NO. 12

Homoinduction: Sender ⇥, onion root tip, variety Texas white. Detector ⊙, root meristem, same variety. Apparatus, inductorium no. 1. Separation, quartz capillary tube and water. Distance, close touch. Time of exposure, 2 hours 30 min. Fixation, Bouin-Allen. Staining, iron haematoxylin, 10 μ section.

(a) Exposed side mitoses	56	62	82	60	28	43	55	40	32	18
(b) Unexposed side mitoses	33	35	32	25	20	21	28	30	18	15
(c) Difference	23	27	50	35	8	22	27	10	14	3
(d) Induction, per cent.	+69	+76	+156	+140	+40	+104	+96	+33	+77	+20

Results: Exposure of an onion root meristem, used as detector against the onion root tip of the same variety used as sender at a distance of 1 mm. for 2 hours 30 minutes, through quartz glass results in definite induction or in an increase in the number of ripe nuclei on the exposed side or in comparison to that of the unexposed side. Average, + 81 per cent.

EXPERIMENT NO. 13

Homoinduction: Sender ⇥, onion root tip, variety Texas white. Detector ⊙, root meristem, same variety. Apparatus, inductorium no. 1. Separation, quartz plate 2 mm. thick from the side. Distance, 1 mm. Time of exposure, 2 hours 30 minutes. Fixation, Bouin-Allen. Staining, iron haematoxylin, 10 μ section.

(a) Exposed side mitoses	46	38	58	60	52	57	62	64	52	51
(b) Unexposed side mitoses	30	31	40	45	40	45	42	48	41	48
(c) Difference	16	7	18	15	12	12	20	16	11	3
(d) Induction, per cent.	+53	+20	+44	+33	+30	+26	+47	+33	+38	+6

Results: Same as in experiments nos. 11C, 11D, 12. Average, + 32 per cent.

EXPERIMENT NO. 14

Homoinduction: Sender ⇥, onion root tip, variety Texas white. Detector ⊙, onion-root meristem, same variety. Apparatus, inductorium no. 1. Separation, quartz. Time of exposure, 2 hours 30 minutes. Fixation, Gibson. Staining, iron haematoxylin, 10 μ section.

(a) Exposed side mitoses 62 30 85 63 80 64 59 58 58
(b) Unexposed side mitoses 44 28 39 45 50 45 36 41 62
(c) Difference 18   2 46 18 30 19 23 17 −4
(d) Induction, per cent. .... +41 +7 +118 +40 +60 +42 +64 +41 −6

Results: Same as in previous experiment. Average induction, + 45.2 per cent.

EXPERIMENT NO. 15

Homoinduction: Sender ⚥, onion-root tip, variety Texas red. Detector ☉, onion-root meristem, same variety. Apparatus, inductorium no. 4. Separation, quartz and air. Time of exposure, 2 hours 30 minutes. Fixation, Bouin-Allen. Staining, iron haematoxylin, 10 μ section.

(a) Exposed side mitoses 72 66 75 80 82 76 61 49 35
(b) Unexposed side mitoses 55 39 45 45 42 44 50 21 20
(c) Difference 17 27 30 35 40 32 11 28 15
(d) Induction, per cent. .... +30 +69 +66 +77 +95 +72 +22 +133 +75

Results: Definite induction. Average, + 71 per cent.

Heteroinduction

It has been stated a number of times before that induction is not limited to the action of a species or variety upon itself, but different biological sources may be used as sender for a given detector. That is, induction by one varietal sender and a different varietal detector is possible. In other words, not only homoinduction but heteroinduction can be produced. To prove this, a set of experiments have been conducted with different plants.

EXPERIMENT NO. 16

Heteroinduction: Sender ⚥, hyacinth root, of unidentified commercial variety. Detector ☉, onion-root meristem, variety Texas red. Apparatus, inductorium no. 1. Separation, quartz capillary tube and water. Time of exposure, 2 hours. Fixation, by Bouin-Allen. Staining, iron haematoxylin, 10 μ section.

(a) Exposed side mitoses 32 56 32 69 35 43 38 65
(b) Unexposed side mitoses 26 32 19 42 30 26 34 30
(c) Difference 6 24 13 27   5 17   4 35
(d) Induction, per cent. .... +23 +75 +68 +64 +16 +65 +11 +11

Results: Definite induction. Average, + 41 per cent.

EXPERIMENT NO. 17

Heteroinduction: Sender ⚥, willow root, unidentified species from Kew Gardens, Long Island, N. Y. Detector ☉, onion root meristem, variety Texas white. Both in inductorium no. 1. Separation, quartz capillary tube and air. Distance, 1 mm. Time of exposure, 2 hours 30 minutes. Fixation, Bouin-Allen. Staining, iron haematoxylin, 10 μ section.

(a) Exposed side 51 43 44 63 50 44 65 58 72 52
(b) Unexposed side 35 41 33 44 33 42 31 43 34 44
(c) Difference 16   2 11 19 17   2 34 10 38   8
(d) Induction, per cent. .... +45 +4.8 +33 +43 +51 +4 +109 +20 +110 +18

Results: Definite induction. Average, + 43.7 per cent.

In studying the cause of ROSSMAN's failure (18) to obtain positive results I conclude that there were two weak points in his technique: (1) the poor centering of roots during the experiments, and (2) improper fixation solution used for the root tips serving as detectors.

The simplest method of detecting energy emanation from different senders of a biological or physical character is the use of yeast culture on agar blocks. This method excludes the necessity of microtome technique, and was worked out by BARON (1). The writer used the micro-incubator placed on the microscope table with quartz object glass, through which induction was made.[4]

The induced yeast culture, after exposure, has been placed on the object glass by means of a platinum loop, fixed and stained. The check control sample from the uninduced (unexposed) culture has been placed by the same method on another object glass, fixed and stained in the same way. The percentage of budding cells has been counted on both object glasses and the difference found. A few figures obtained are shown below.

EXPERIMENT NO. 78

Heteroinduction: Sender ≠, *Bacillus anthracoides*, bouillon culture. Detector ⊙, yeast culture, 12 hours glucose bouillon agar. Apparatus, micro-incubator. Temperature, 32° C. Separation by quartz, 1 mm. thick. Distance, 2 mm. Time of exposure, 2 hours.

(a) Exposed culture budding, per cent.	. 75
(b) Unexposed culture budding, per cent.	41
(c) Difference	. 34
(d) Induction, per cent.	+83

Results: Presence of induction shown.

EXPERIMENT NO. 79

Heteroinduction: Sender ≠, *Bacillus anthracoides* culture. Detector ⊙, yeast culture, *S. cerevisiae*. Apparatus, celluloid and quartz plates in a thermostat. Temperature, 32° C. Distance, 2 mm. Time of exposure, 2 hours.

(a) Exposed culture budding, per cent.	40
(b) Unexposed culture budding, per cent.	21
(c) Difference	19
(d) Induction, per cent.	+90

Results: Presence of induction shown.

EXPERIMENT NO. 75A

Heteroinduction: Sender ≠, onion root, variety Texas red. Detector ◯, yeast culture. Apparatus, micro-incubator. Separation, 1 mm. quartz object glass. Temperature, 32° C. (89° F.). Distance, 3 mm. Time of exposure, 2 hours 30 min.

[4] Later on, a special inductorium was constructed, consisting of a quartz plate, diaphragm, heating element, and a glass cylinder for yeast culture. Optimum temperature was controlled by a rheostat.

(a) Exposed culture budding, per cent. 65
(b) Unexposed side budding, per cent. 41
(c) Difference 24
(d) Induction, per cent. .... +59
Results: Definite induction shown.

My experiments confirm the existence of energy emanation during the cell division processes in four biological objects: onion root, hyacinth root, willow root, and bacteria. The energy is in the form of radiation, penetrating through quartz glass, and stimulating the tempo of the cell division process of the onion-root tissues and yeast cell colony. Tissues of different plants, yeast or bacteria cells can affect each other as well. The effect is noted at a distance.

The dividing cell has proven to be as good a detector of energy as the most sensitive physical instruments, and may perhaps be even more sensitive. The sensitiveness of a living organism and its use as a most delicate instrument recalls the experiments of PACKARD (15) with *Drosophila* eggs, used for quantitative determination of Roentgen units.

The existence of a specific energy emanation in a living cell may be of greater significance than it appears at the present, and should be studied carefully. Experiments thus far conducted show definitely that it has a stimulating, if not a specific, effect on the living cell or at least on its nuclei to the extent of inducing their division directly or indirectly. The theory of M-Rays (mitogenetic rays) as a working hypothesis will stimulate research in the field of experimental cytology, general physiology, as well as in the field of genetics, particularly in the study of gene activity. At any rate, the investigation of the physical side of the problem will thus be promoted.

The organism as a whole has the same functional elements as a cell has in miniature. The organism is a field of activity, as it were, of a tornado of chemical and physical forces. Whereas the chemical side has been studied carefully and some questions already have been answered, the physical side of the processes in the living organism has not yet been an object of intensive study, owing to the fact that scientists did not possess the delicate instruments which are now coming into use by modern physicists: thermocouples, photoelectric cells, hydrogen-ion concentration apparatus, etc.

We know at present how powerful an influence X-Rays, radium, and even temperature, phenomena of a physical category, have on the living organism. These forces, in the hands of a scientist, have already been shown to produce considerable changes in the most hidden parts of an organism, *viz.*, the germ-plasm. All these forces are produced artificially and

outside the organism.   We know very little, however, about the existence of these forces in the living organism, and they are wondered at when they are occasionally discovered.   Can an organism be a producer of heat, light, or electricity?   The answer is—yes, for it has been readily detected.   But can the organism be a source of ultra-violet rays, X-Rays, or other "unusual" radiations?   We are still in doubt about this.   Many of us still answer—"impossible."

From the studies of M-radiation we know now that the *cells* possess the ability of some energy emanation which has been likened to ultra-violet radiation of a definite wave length.   The source of this energy emanation has been shown to be the property of living cells the activity of which stimulates some of the life processes.[5]

There are many possibilities that this theory will have a far-reaching application in the field of genetics.   It is of interest to note that of the 62 papers presented at the section of genetics at the New York meeting of the A. A. A. S. in 1928, 12 were devoted to the action of X-rays.[6]

The induction by M-Rays (mitogenetic rays), biophysical forces, at a distance, effects as far as we know at present, the division of nuclei and of chromosomes *in toto*.   The possibility of a selective effect on parts of chromosomes is not excluded.   The X-ray effect, if not selective, may at any rate, according to one theory, destroy a complete molecular complex comprising a gene, thereby changing the balance.   The result is the dislocation of other molecular parts in the field created by X-rays, and destruction of this group.   According to another theory, X-rays rearrange the molecular structure of a part of a chromosome or of a gene.

In all experiments with the breeding of *Drosophila* under varying temperatures, from 19° C. to 30° C., commercial yeast cultures belonging

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[5] We can now almost suspect the close connection between the origin of vitamins and activity of this radiation.   Both vitamins and radiation are essential in substances used by organisms as food.   Vitamin D, as antirachitic, has been shown to depend somewhat on ultra-violet radiation.   Here is a wide horizon for fruitful research and explanations.

[6] The molecular components of chromosomes, chromomeres, chromioles and even genes may be considered as the specific structural centers of organic function.   We may assume that each molecular unit is produced from similar molecular units with no change in the chemical composition and with no changes in the physical properties.   The chemical homogeneity of the molecular units of the chromosome parts is the basic foundation of heredity.   The production of more molecular units of the same character is only possible by longitudinal division of the chromosome (*"omnis molecula ex molecula"*).   This comprises the equal division of every molecular component.   Thus it repeats all the complex characteristics of a single gene complex comprising a chromosome.   The biochemical and bio-physical activity of a single group of molecular units, not more than the size of octokaidekapeptid, or about 0.01 μ, in bunches close together, may be identified with the activity of a gene which is conceived to be a complicated center of combined activity of a single group of molecular units.

to the strain of *Saccharomyces cerevisiae* were used as food. According to some statements, the maximum multiplication and maximum number of mutations occur at a temperature close to 30° C. I should like to point out the fact that the optimum of multiplication of yeast cultures of this particular race occur at this same temperature or about 28°–29° C.

Is the change in temperature itself the *only cause* of the increase of multiplication and mutations of *Drosophila?* Or is it the result of radiation emanating from the yeast as well? We have no proof to confirm this, but it would be of interest to use different species of yeasts whose optimum multiplication occurs at different temperatures from the above (below 19° or above 29° C.). A study of the progeny of *Drosophila* and of the number of mutations occurring under these new conditions may show some interesting results.

The source of M-Rays (mitogenetic rays) in a living cell has not been definitely located, but it has been found already that not all the tissues of plants and animals possess this peculiarity in equal measure. The possibilities of the location of this activity in the nuclei or chromosome substances is not to be excluded. If it should prove to be the case that the radiations arise from the nuclei or chromosomes, a new page in the history of the study of gene activities will be opened. At present, however, we only know that there is a definite connection between the intensity of the metabolic processes and the emanation of M-rays.

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LITERATURE CITED

1. Baron, M. A. Über mitogenetische Strahlung bei Protisten. Arch. Entw. Organ. **108**: 619–628. 1926.
2. ——————. Bakterien als Quellen mitogenetischer (ultra-violetter) Strahlung. Zentralb. Bakt. und Parasitenkunde **73**: 373–379. 1928.
3. Frank, G., and Salkind, S. Die Quellen der mitogenetischen Strahlung im Pflanzenreich. Arch. Entw. Organ. **108**: 596–608. 1926.
4. Gurwitsch, A. Les mitoses de croissance embryonnaire exigent-elles une stimulation extracellulaire? Compt. Rend. Soc. Biol. **83**: 1552–1553. 1920.
5. ——————. Les problèmes de la mitose et les rayons mitogéniques. Bull. d'Histol. Appl. **1**: 486–497. 1924.
6. ——————. Die Natur der specifischen Erregers der Zellteilung. Arch. Mik. Anat. **100**: 11–40. 1924.
7. ——————. Das Problem der Zellteilung physiologisch betrachtet. Monograph Gesamtgeb. Physiol. Pflanz. und Tiere. No. 2. Springer. Berlin. 1926.

8. GUTTENBERG, H. VON. Die Theorie der mitogenetischen Strahlen. Biol. Zentralbl. **48**: 31–39. 1928.

9. ——————. Schlusswort zur Arbeit von B. Rossmann. Arch. Entw. Organ. **113**: 414–418. 1928.

10. HABERLANDT, G. Zur Physiologie der Zellteilung. Sitzungsber. preuss. Akad. Wiss. Berlin. **20**: 322–348. 1919.

11. ——————. Über Zellteilungshormone und ihre Beziehungen zur Wundheilung, Befruchtung, Parthenogenesis, und Adventorembryologie. Biol. Zentralbl. **42**: 145–172. 1922.

12. ——————. Wundhormone als Erreger von Zellteilungen. Beitr. allg. Bot. **2**: 1–53. 1921.

13. KISLIAK-STATKEWITSCH, M. Das mitogenetische Strahlungsvermögen des Kartoffelleptoms. Arch. Entw. Organ. **109**: 283–286. 1927.

14. MAGROU, J., and MAGROU, M. Recherches sur les radiations mitogénétiques. Bull. d'Histol. Appl. **4**: 253–262. 1927.

15. PACKARD, CHARLES. The measurement of quantitative biological effects of X-rays. Jour. Cancer Res. **10**: 319–339. 1926.

16. RAWIN, W. Weitere Beiträge zur Kenntnis der mitotischen Ausstrahlung und Induktion. Arch. Mikr. Anat. **101**: 53–61. 1924.

17. REITER, T., and GABOR, D. Zellteilung und Strahlung. Springer. Berlin. 1928.

18. ROSSMANN, BRUNO. Untersuchungen über die Theorie der mitogenetischer Strahlung. Arch. Entw. Organ. **113**: 346–406. 1928.

19. WAGNER, N. Über den von A. GURWITSCH entdeckten spezifischen Erreger der Zellteilung (mitogenetische Strahlen). Biol. Zentralbl. **47**: 670–678. 1927.

20. WENT, F. A. F. C. Plant movements. Proc. International Congress of Plant Sciences **1**: 1–12. 1929. Geo. Banta Pub. Co.

TOXICITY OF SOME ALIPHATIC ALCOHOLS

WALTER S. EISENMENGER

(WITH SIX FIGURES)

Previous investigations have revealed the importance of certain ions for plant life, and the use of mixtures of salts in aqueous solution containing these ions has led to a conception of a favorable medium for complete plant development.

Nutritive value of solutions

The researches of KNOP (**8, 9**) and BIRNER and LUCANUS (**1**) showed that higher plants require nitrogen, sulphur, phosphorus, calcium, magnesium, iron and potassium ions in an aqueous solution about their roots.

More recently McHARGUE (**19**) has shown that in the total absence of manganese plants do not develop normally. JOHNSTON and DORE (**7**), SOMMER and LIPMAN (**31**), SOMMER (**30**), and others have shown that boron is essential. Additional evidence [SOMMER and LIPMAN (**31**)] has indicated that zinc is needed.

The first investigation that led to a systematic testing of logically complete series of salt proportions was by TOTTINGHAM (**32**). This work, in addition to that of SHIVE (**29**) and others, has indicated that best growth can be secured with a solution having an osmotic value of 0.25 to 2.50 atmospheres. Very concentrated solutions may exert a physical influence in retarding the absorption of water by the plants, as well as a toxic effect, if their components are not properly balanced; extremely dilute solutions though practically non-toxic may fail to supply the essential chemicals rapidly enough for best growth.

Several improvements in solution-culture technique have been given attention by TRELEASE and FREE (**33**), and TRELEASE and LIVINGSTON (**34**); of selecting seeds for uniformity of weight by TRELEASE and TRELEASE (**35**).

LOEB (**12, 13, 14, 15**) was the first to describe the antagonistic action of salt solutions. He was the first to discuss clearly the difference between balanced solutions and nutrient solutions. LOEB discovered that young fish (*Fundulus*) soon die if placed in a solution of NaCl of the concentration in which this salt is contained in sea water. When KCl and $CaCl_2$ were added to the solution in the right proportions the fish could live indefinitely.

Antagonism was considered by LOEB to depend upon an action of both salts and the egg membrane, whereby the membrane becomes nearly impermeable to both salts. The toxicity of each salt in simple solution was assumed to be due to its diffusion through the membrane.

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OSTERHOUT (21) emphasizes the fact that the injurious effects of single salt solutions cannot be due to the lack of nutrients, for distilled water is less harmful than simple solutions. OSTERHOUT (22, 23, 24) calls attention to the fact that in order to know the degree of antagonism, it is necessary to know the additive effect—the total toxic effect that the solution would exert if no antagonism existed and each simple solution exerted its toxic effect independently. By comparing the additive effect with the effect in a mixed solution it can be seen whether the toxicity of the two salts is unaltered (the same as the additive effect), whether the toxicity is diminished (less than the additive effect), or whether the toxicity is increased (greater than the additive effect).

With respect to the toxicity of the simple monohydroxy alcohols little of a quantitative nature has been reported. RICHARDSON (27, 28) states, regarding their toxicity, that they change in character regularly as the carbon chain increases in length. He found the death point for *Medusa* in ethyl alcohol to be at a concentration of from 0.03 to 0.1 per cent.

HODGE (6) found that when ethyl alcohol is present in yeast cultures to the extent of 0.01 per cent., 992 cells of yeast were produced instead of 2061 under normal conditions. In this instance there was retardation of cell division due to the presence of alcohol.

RAUBER (26) used ethyl alcohol and found that roots of *Impatiens* were killed in a 5 per cent. ethyl alcohol solution. In a 20 per cent. solution *Pinus larix* turned yellow, and a 5 per cent. solution destroyed fungi when applied to soil around them.

MALTAUX and MASSART (17) reported that in the case of *Paramecium* there was an increase in cell division when treated with dilute ethyl alcoholic solutions. This characteristic was later lost.

Alcohols have been regarded as protoplasmic poisons. However, DANIEL (3), who used two strains of *Stentor,* reports that these organisms can acclimate themselves to a degree in very dilute solutions of ethyl alcohol. In a 1 per cent. solution they lived as long as one week in a favorable condition. A 2 to 3 per cent. solution produced death after a period of six and two hours respectively. In a 4 per cent. solution they always died. They were not resistant to an 8 per cent. solution but died in eighty to one hundred seconds. In a 1 per cent. solution of methyl alcohol *Stentor* became acclimated to the medium. From the data cited, it seems that low concentrations of alcohol in these instances constituted a lethal dose for both plants and lower animals.

For higher forms of animal life, MUNCH and SCHWARTZE (20) found the average lethal dose for rabbits to be as follows: Cubic centimeters of alcohol per kilo weight: Methyl 18, ethyl 12.5, normal propyl 3.5, isopropyl

10, normal butyl 4.25, isobutyl 3.75, secondary butyl 6, tertiary butyl 4.5, isoamyl 4.25, secondary amyl 3.5, tertiary amyl 2.5. In this instance the order of relative toxicity is to a large degree as it is in plant life.

Criteria of growth

In this investigation two criteria of growth were employed: (1) elongation of roots of germinating soy-beans; (2) the ash of the shoot (stem and leaf). The initial behavior of germinating beans involves less complexity within as well as without the organism than is involved in later phases of growth. In the initial stages of growth the plants are well supplied with organic food from the seed, and the supply of salts is sufficient to afford protection from a condition of starvation of roots grown in distilled water. The protoplasm of the roots appears to be readily accessible to the toxic agent.

The three salts, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$, were chosen for the preparation of the control solution because of their importance in supplying ions needed in normal growth in plants.

In an extended investigation SHIVE (29) found that wheat plants supplied with these three salts grew during the first four weeks after germination as well as they did in solutions containing a greater number of salts.

In this investigation a study was made of the toxicity of the following alcohols: methyl, ethyl, normal propyl, isopropyl, normal butyl, isobutyl, secondary butyl, tertiary butyl, normal amyl, isoamyl, and normal hexyl, and also that of calcium nitrate. The concentration of each alcohol or salt ranged from 0.0012 M. to 0.06 M. (2, 5, 15, 30, 50, 85, 98, and 100 per cent. of 0.06 M.). Tests to show possible antagonism were made by using mixed solutions, each containing a normal alcohol and calcium nitrate—namely, methyl + $Ca(NO_3)_2$, ethyl + $Ca(NO_3)_2$, normal propyl + $Ca(NO_3)_2$, normal butyl + $Ca(NO_3)_2$, normal amyl + $Ca(NO_3)_2$. With each concentration, ten sets of molecular proportions of alcohol and calcium nitrate were used as follows: 0 + 100, 2 + 98, 5 + 95, 15 + 85, 30 + 70, 50 + 50, 70 + 30, 85 + 15, 98 + 2, 100 + 0.

Methods

The methods employed were essentially the same as those described by TRELEASE and TRELEASE (36), and by EISENMENGER (4). A pure line of soy-beans (Virginia) was used, secured through the courtesy of Professor J. E. METZGER, of the University of Maryland. The seeds were germinated on moist filter-paper in glass culture dishes in a dark room.

For each culture two Pyrex beakers (tall form, without spout) were used. The smaller beaker was of 300-cc. capacity, the larger of 600-cc. capacity. Over the top of the small beaker was stretched a piece of paraffined mosquito netting, which was secured below the rim by a ligature of

paraffined linen thread. The smaller beaker was placed inside the larger one, and the culture solution was poured in until the liquid levels inside and outside the smaller beaker were even at its top.

When the average length of the primary roots of the seedlings was about 8 mm. selected seedlings were placed on the mosquito netting so that the roots dipped into the culture solution. Duplicate cultures, each of twenty-five plants, were used for each experimental solution. The cultures were placed in a dark room, and the seedlings were allowed to grow until the primary roots of the control culture had acquired a length of about 95 mm. or until these roots had elongated 87 mm. (95 mm. minus 8 mm).

The length of the primary root of each individual plant was then recorded and the average computed. From this average was deducted the average length of the roots at the time when the seedlings were taken from the germination chamber.

This difference constituted the average root elongation value for the culture. The growth data presented in this paper are relative values. Each relative growth value was obtained by dividing the average elongation of a given culture by the average elongation of the control culture and multiplying this quotient by one hundred.

Another criterion of growth was the relative ash content of the shoots of the seedlings. The shoots were cut from the seed when the roots had made new growth of about 87 mm. (95 mm. minus 8 mm.). The shoots were then placed in weighed porcelain crucibles and burned completely to ash in an electric furnace and weighed on an analytical balance. Here again, as in the root elongation data presented, values are relative. Each relative growth value was obtained by dividing the average ash weight of a given culture by the average ash weight of the control culture and multiplying this quotient by one hundred.

The average temperature during the growth of the seedlings was 21° C. The average time required for the roots of the control solutions to acquire an additional length of 87 mm. was 98 hours. Four control cultures of 25 plants each were used. Also, two distilled water cultures of 25 plants each were included in each series. The composition of the control solutions was as follows: 0.02 M. KH_2PO_4, + 0.02 M. $Ca(NO_3)_2$, and 0.02 M. $MgSO_4$.

The water used was obtained from a Barnstead still. The salts were "Baker's analyzed," and the alcohols were Eastman's. The latter conformed in boiling point to the standards usually ascribed to them.

Results of tests with single alcoholic solutions and single salt solutions of calcium nitrate

The relation between root elongation rates and volume-molecular concentrations of single normal alcohol solutions and of single solutions of calcium nitrate are shown in the graphs of figure 1.

The abscissae represent volume-molecular concentrations, and the ordinates represent growth rates expressed as percentages of the root elongation in the three-salt standard or control solution. In each case all of the observed points are shown, and the curve represents what appears to be the general trend of the points. It will be observed that the seven graphs differ greatly in form.
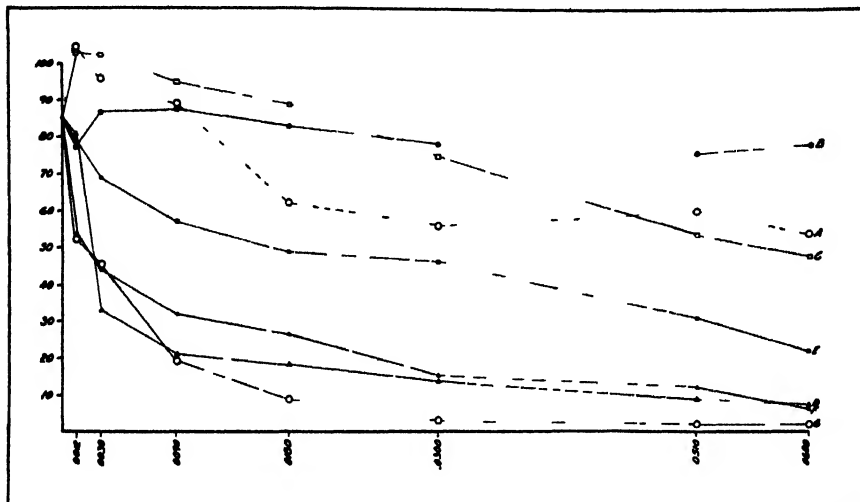

FIG. 1. Elongation of soy bean roots in simple solutions of normal alcohols, and calcium nitrate. Ordinates represent percentage of elongation for standard solutions; abscissas represent volume-molecular concentrations. A. Calcium nitrate; B. Methyl; C. Ethyl; D. N propyl; E. N butyl; F. N amyl; G. N hexyl.

SOLUTIONS OF CALCIUM NITRATE

The graph for $Ca(NO_3)_2$ in figure 1 rises from 0 molar to 0.0012 M. From 0.0012 to 0.03 M. there is a decided slope downward, and again a slight rise at 0.051 M. The unexpected growth at 0.0012 M. seems to indicate that for soy-beans in their early stages of growth, $Ca(NO_3)_2$ at very low concentrations has a stimulating influence. Wheat seedlings do not show this increased growth effect at low concentrations (4).

SOLUTIONS OF METHYL ALCOHOL

Solutions of this alcohol are slightly more toxic than distilled water. At the lowest concentrations it is slightly less toxic than distilled water and at higher concentrations, more toxic than distilled water.

SOLUTIONS OF ETHYL ALCOHOL

Ethyl alcohol in its physiological action differs from other alcohols in that at very low concentrations it exerts a decided stimulating influence. It

allows a growth rate at 0.0012 M. greater than does a standard solution. As the concentration increases from 0.0012 M., there is a gradual increase in toxicity until it reaches the maximum strength used, 0.06 M.

SOLUTIONS OF NORMAL PROPYL ALCOHOL

Normal propyl alcohol seems to be one of the most toxic alcohols. A solution of 0.003 M. concentration allows root elongation equal to 33 per cent. of the growth rate in a standard solution. The toxicity gradually increases with concentration until, at 0.06 M., the growth is equivalent to only 7 per cent. of the growth attained in a standard solution.

SOLUTIONS OF NORMAL BUTYL ALCOHOL

N-butyl alcohol is more toxic than methyl or ethyl alcohol throughout all concentrations, and less toxic than is N-propyl alcohol. It increases in toxicity with increased concentration.

SOLUTIONS OF NORMAL AMYL ALCOHOL

N-amyl alcohol is approximately as toxic as N-propyl alcohol. At concentration 0.0012 M. it is slightly more toxic, and at concentrations 0.003 M. to 0.06 M. it is slightly less toxic than normal propyl alcohol at the same molar concentration.

SOLUTIONS OF HEXYL ALCOHOL

Throughout all concentrations used except one (0.003 M.) this is the most toxic alcohol. At this concentration, propyl alcohol is slightly more toxic. This difference is so small, however, as to permit the statement that hexyl alcohol is the most toxic alcohol used. The roots in solutions of 0.03 M. to 0 06 M. seemed to be killed soon after being immersed in the solution.

SOLUTIONS OF ISOPROPYL ALCOHOL

There is little difference in the growth-retarding influence of isopropyl alcohol and methyl alcohol. From concentrations 0.0012 M. to 0.0018 M. this alcohol seems to be approximately as toxic as distilled water. At concentrations higher than 0.0018 M. it is slightly more toxic than distilled water. It is one of the least toxic alcohols.

Comparisons of growth in different alcohols

It is interesting to institute comparisons between the physiological action of some of the alcohols that are similar in structure. Thus, comparing two alcohols of the same number of carbon atoms, propyl and isopropyl, it will be observed that they represent the extremes in their physiological action—the former exceedingly toxic, the latter relatively inert.

In a general way one might classify methyl alcohol and ethyl alcohol together in a group whose physiological action is somewhat alike. At very low concentrations they seem to have a stimulating effect, and at higher concentrations they are less toxic than are the other alcohols. In a group showing high toxicity may be included normal propyl, normal amyl, and normal hexyl alcohol. The general outline of the growth curves seems to indicate that these produce similar toxicity, though not all to the same high degree. Normal butyl alcohol stands midway between these two classes in that it is moderately toxic.

BUTYL ALCOHOLS

It has been stated by McCOLLUM (18) that the toxicity of the alcohols increases with the increasing number of carbon atoms in the molecule, and that the normal alcohols are more toxic than those alcohols in which the atoms are not represented in a straight chain in the molecule.

In fig. 3-B are presented the root elongation curves of the soy-bean in four butyl alcohols. At a low concentration, as 0.003 M., the root elongation in solutions of normal, secondary, and tertiary butyl alcohols is about the same. From 0.003 M. to 0.06 M. there is a decided increase in toxic effects of all three alcohols. This increase is most rapid in the case of normal butyl alcohol, less so in solutions of tertiary butyl alcohol, and least so in the case of the secondary butyl alcohol. Isobutyl alcohol throughout most of the different concentrations exerts the greatest toxic effect of all the butyl alcohols. However, at the low concentration of 0.0012 M. this seemingly toxic alcohol has a stimulating effect. This is of interest, since it illustrates the principle frequently stated in physiological literature, that dilute solutions of a substance which is toxic in higher concentrations may be expected to have stimulating effects.

AMYL ALCOHOL

Among the alcohols thus far discussed no two alcohols containing the same number of carbon atoms per molecule resemble each other more closely with respect to their influence on soy-bean seedling growth than do the two amyl alcohols—normal and isoamyl (fig. 3-A). At low concentrations the normal alcohol is somewhat more toxic than is isoamyl alcohol.

GENERAL ORDER OF GROWTH EFFECTS

At the maximum concentration, 0.06 M., the order of toxicity from least to most toxic is as follows: methyl, isopropyl, secondary butyl, tertiary butyl, ethyl, normal butyl, isobutyl, isoamyl, normal propyl, normal amyl, normal hexyl.

At a concentration of 0.03 M. the order of increasing toxicity is as follows: isopropyl, methyl, ethyl, secondary butyl, tertiary butyl, normal butyl, isobutyl, isoamyl, normal amyl, normal propyl, normal hexyl.

TABLE I

GROWTH IN SOLUTIONS OF ALCOHOLS AND IN CALCIUM NITRATE AS INDICATED BY RELATIVE ROOT ELONGATION

MOLECULAR	AVERAGE OF INDIVIDUAL TESTS OF 50 SEEDLINGS EACH											
	METHYL	ETHYL	NORMAL PROPYL	ISO-PROPYL	NORMAL BUTYL	ISO-BUTYL	SECONDARY BUTYL	TERTIARY BUTYL	NORMAL AMYL	ISOAMYL	NORMAL HEXYL	CALCIUM NITRATE
0.0012	78	104	81	81	79	98	87	79	54	71	54	104
0.0030	74	103	33	86	69	83	80	72	44	63	45	96
0.0090	88	95	21	84	57	42	80	71	32	53	20	88
0.0180	83	89	18	86	49	30	69	60	26	30	9	62
0.0300	78	75	14	81	46	27	69	53	15	18	3	56
0.0510	76	54	9	72	31	20	61	50	12	11	2	60
0.0600	78	48	7	75	22	20	39	54	6	9	2	54

The variation from minimum to maximum root elongation is greatest in the case of isobutyl alcohol, ranging from 20 per cent. (of the root elongation in a control solution) at a concentration of 0.06 M. to 98 per cent. at a concentration of 0.0012 M. In methyl alcohol this variation is least, ranging only from 76 per cent. at 0.051 M. concentration to 88 per cent. at 0.009 M. Thus the seedlings grown in solutions of methyl alcohol are least affected by variation of molar concentration, and seedlings grown in solutions of isobutyl alcohol are most affected by variations of concentration. The growth data upon which the foregoing discussion is based are presented in table I.

Concentration and toxic action

An examination of the literature shows that a relatively simple relation between concentration and toxic action has been derived from some studies. Thus CHICK (2), working on the effect of disinfectants on the period of bacterial survival was able to state the relation in simple mathematical terms. The process of disinfection was found to obey the laws of a monomolecular reaction, characterized by a definite velocity constant; and a study of the velocity constants for a wide range of concentrations of the disinfectants showed that the velocity of disinfection is a power function of the concentration.

Similar relations have been reported by KRONIG and PAUL (10), PAUL, BERNSTEIN and REUSS (25), and FALK and WINSLOW (5). The same idea is involved in the conclusion of LeRENARD (11), who reported that the degree of toxicity of any solution may be calculated if we know the coefficient of toxicity of the salt in question and its concentration in solution. Similarly, OSTERHOUT (23) states that in most cases equally toxic solutions, if both solutions are diluted to the same degree, will remain equally toxic. It should be observed, however, that the results secured in the present study show that the order of toxicity of several alcohols is not always constant, but varies with the concentrations used.

In the case of the alcohols and calcium nitrate, a mathematical expression has been derived from the data for each compound, which may be used to calculate the approximate relative growth at any concentration falling within the limits of those used—0.0012 M. to 0.06 M. Thus in the exponential approximation by method of least squares the following items are indicated; let a = average (of relative root length or average of relative ash content); m = the molar concentration; e = Napierian base.

For example, we may consider secondary butyl alcohol at a concentration of 0.03 M. The expression for this compound when root length is considered, is: $a = 82.8e^{-6m}$. Now by the theory of logarithms, log a = log 82.8e – 6m log e, but in the Napierian system log e equals unity. Therefore

log a = log 82.8 − 6m or log a = log 82.8 − 6 (0.03) and by simplifying, log a = 4.416 − 0.18 or 4.236.   The number corresponding to this natural logarithm is 69.   In this instance the calculated value is identical to that representing the relative root length at the concentration of 0.03 M.

The following are the values thus calculated:

	FOR ROOT LENGTH	FOR ASH CONTENT
1 Methyl	$a = 80.3e^{-.5m}$	$a = 48.9e^{-1.2m}$
2 Ethyl	$a = 108.5e^{-13.4m}$	$a = 70.7e^{-12.5m}$
3. Normal propyl	$a = 41.4e^{-31.6m}$	$a = 36.7e^{-21.2m}$
4. Isopropyl	$a = 85.5e^{-2.5m}$	$a = 57.6e^{-2m}$
5. Normal butyl	$a = 74.1e^{-18.8m}$	$a = 51.6e^{-24.9m}$
6. Isobutyl	$a = 69.1e^{-24.5m}$	$a = 47e^{-16.7m}$
7. Secondary butyl	$a = 82.8e^{-6m}$	$a = 50.5e^{1.1m}$
8. Tertiary butyl	$a = 73.1e^{-6.7m}$	$a = 50e^{6.3m}$
9. Normal amyl	$a = 47.6e^{-32.5m}$	$a = 45.3e^{-34.4m}$
10. Isoamyl	$a = 66.7e^{-35.7m}$	$a = 70e^{-36.6m}$
11. Normal hexyl	$a = 37.4e^{-57.3m}$	$a = 30.3e^{-62m}$
12. Calcium nitrate	$a = 92.2e^{-16.2m}$	$a = 77.6e^{-5.3m}$

Most of the values calculated compare favorably with the actual comparative measure of growth.   However, in the case of the most toxic compounds, as hexyl alcohol, and the least toxic compounds, as methyl alcohol and isopropyl alcohol, calculations may be considered as less applicable.   The curves representing the toxicity of these compounds tend to flatten out at the low concentrations for the less toxic compounds, or at the higher concentrations for the more toxic compounds.

COMPARISON OF MOLECULAR CONCENTRATIONS PRODUCING EQUAL
GROWTH RATES

If any pair of curves discussed had the same slope, it would signify the existence of a constant ratio between the molecular concentrations of two solutions producing equal growth rates.   Since the slopes are different, it is of interest to see to what extent the ratios differ, as indicated by table II.

PHYSICAL CONSTANTS AS RELATED TO TOXICITY.—The results of these experiments did not seem to justify any statements regarding a relationship between such physical constants as density, specific heat, refractive index, molecular refraction, viscosity, and the toxicity of the alcohols used.

RELATION OF TOXICITY TO NUMBER OF CARBON ATOMS IN THE MOLECULE OF ALCOHOL.—Only by making an exception of normal propyl alcohol can it be stated that the toxicity of normal alcohols increases with increase of molecular weight. Incidentally, normal propyl alcohol is the only alcohol used, which is miscible in all proportions with both oil and water.

TABLE II

Molecular concentrations of single alcohol solutions required to produce various equal root elongations

RELATIVE GROWTH RATE	MOLECULAR CONCENTRATIONS OF SINGLE ALCOHOL SOLUTIONS										
	METHYL	ETHYL	N PROPYL	ISOPROPYL	N BUTYL	ISOBUTYL	SECONDARY BUTYL	TERTIARY BUTYL	N AMYL	ISOAMYL	N HEXYL
80	0.0258	0.0258	0.0012	0.0318	0.0012	0.0036	0.003	0.0012		0.0006	
70		0.0354	0.0018		0.0030	0.0048	0.0174	0.0096	0.0006	0.0012	0.0006
60		0.0450	0.0018		0.0078	0.0066	0.0552	0.018	0.0012	0.0048	0.0012
50		0.057	0.0024		0.0174	0.0078		0.051	0.0018	0.0102	0.0024
40			0.0030		0.0390	0.0108			0.0048	0.0144	0.0042
30			0.0048		0.0522	0.018			0.0120	0.018	0.0066
20			0.0120			0.051			0.0246	0.0282	0.009
10			0.048						0.0540	0.0552	0.0174

Ash of shoots

The method of weighing the ash of the stems was adopted to furnish another criterion for determining growth rates. It was not anticipated that the growth rate as judged by root elongation would correspond precisely with the growth rate as measured by the ash content of the stems of the same plant. But as the alcohols themselves furnish no ash constituents to the plant, this criterion of growth would, in a degree, give one an idea as to how much of the inorganic constituents were furnished by the seeds to the stem during a short growing period.

It was found by comparison that the more toxic alcohols inhibit the transportation of plant nutrients from the seed to the stem, and that the ash content of the shoots of seedlings was a fair measure of growth.

The average elongation of seedlings grown in distilled water was 85 per cent. of the average elongation of seedlings grown in a control solution, and the average ash content of shoots grown in distilled water was 52 per cent. of the ash content of shoots grown in a control solution. These data alone reveal the fact that the graphs representing root elongation and the graphs representing ash content of shoots cannot run parallel.

Figure 2 represents the relation between growth rates (as measured by ash content) and volume-molecular concentration of six normal alcohols, and of a single salt solution of calcium nitrate. The order of toxicity of the different alcohols throughout most of the concentrations is practically the same as the order illustrated in fig. 1, where root elongation is plotted against volume-molecular concentration. One of these exceptions is the case of normal propyl alcohol, which by ash content measurement seems slightly less toxic than amyl alcohol at high concentrations. In the case of the root-elongation measurements the former is seen to be the more toxic of the two.

Even more pronounced is the difference in the configuration of the ash and elongation curves for $Ca(NO_3)_2$ (figs. 1 and 2). In the case of ash measurement the curve falls far more slowly than does the root elongation curve (fig. 2), where volume-molecular concentration is plotted against root elongation. In the former figure, the maximum concentration of $Ca(NO_3)_2$ does not appear to exert as much toxic influence as does distilled water. It seems that solutions of salts like $Ca(NO_3)_2$ must contribute directly to the ash content of the seedlings in early periods of growth. However, it is evident that in solutions of high concentration of $Ca(NO_3)_2$, relatively less ash is transported to the seedlings than in cases where smaller amounts of the salt are present.

When the seedlings are grown in various $Ca(NO_3)_2$ solutions, the measure of growth, as indicated by root elongation, does not seem to bear a favorable resemblance to the growth as indicated by the relative ash content.
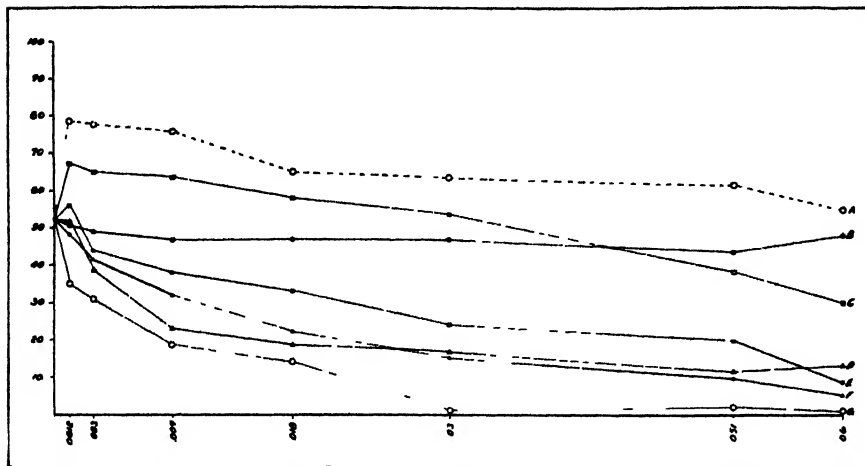
FIG. 2. Ash of soy-bean shoots grown in simple solutions of normal alcohols and calcium nitrate. Ordinates represent percentages of ash for standard solution; abscissas represent volume-molecular concentrations. A. Calcium nitrate; B. Methyl; C. Ethyl; D. Ethyl; E. N butyl; F. N amyl; G. N hexyl.

SOLUTIONS OF PROPYL ALCOHOLS

The relation of growth rate (as measured by relative ash content) and volume-molecular concentration of single solutions of normal propyl and isopropyl alcohol are shown in the graphs of fig. 4-C. The general directional outline of each curve is not greatly different from that where the relation of root elongation to volume-molecular concentration is represented (fig. 3-C). However, as the initial point (distilled water) in each graph is lower than the initial point where the relationship of root elongation to volume-molecular concentration is shown, the curves representing measurement with respect to ash content are not as far apart.

SOLUTIONS OF BUTYL ALCOHOLS

In comparing the toxicity of the different butyl alcohols it is found that the order of increasing toxic properties is as follows: secondary butyl alcohol, tertiary butyl alcohol, normal and isobutyl alcohol. The last two named have approximately the same degree of toxicity. In a general way it may be stated that the butyl alcohols as a class are of moderate toxicity as compared with the slightly toxic alcohols, methyl, ethyl, and isopropyl, and the extremely toxic alcohols, normal propyl, normal amyl, isoamyl, and normal hexyl (fig. 4-B).

SOLUTIONS OF AMYL ALCOHOLS

In fig. 4-A the relationship between gram-molecular volume and ash content of seedlings grown in the two different alcohols, amyl and isoamyl,
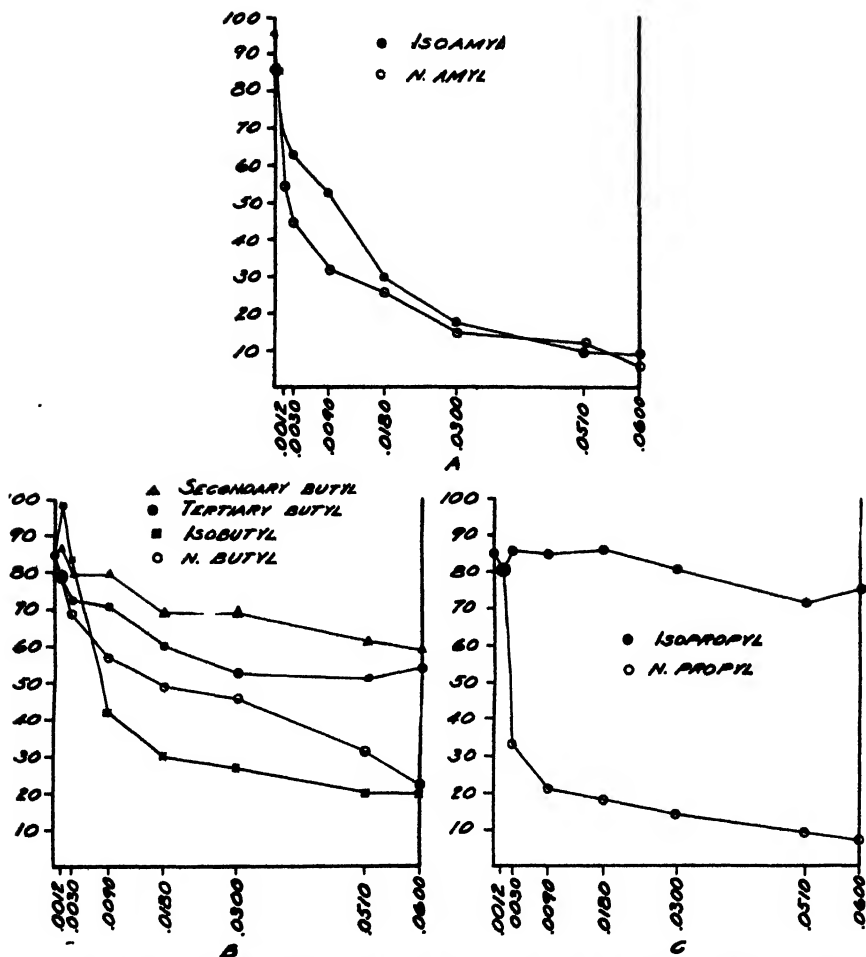
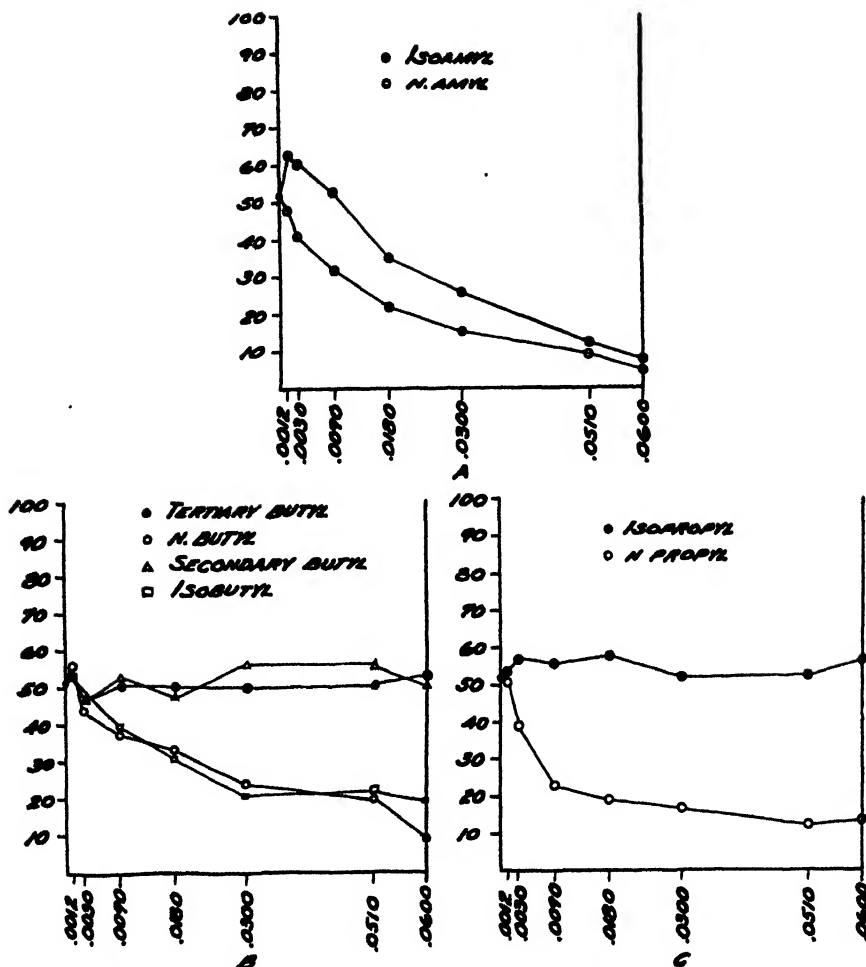FIG. 3.   Elongation of soy-bean roots in simple solutions of eight different alcohols.
Ordinates represent percentages of elongation for standard solution;
abscissas represent volume-molecular concentrations.

is indicated.   Again the normal alcohol is the more toxic, as in the case of
the propyl alcohols.   However, the difference in the case of the amyl alco-
hols is not so pronounced.   At high concentrations there is little difference
in toxicity.   There is a marked similarity in outlines of curves as illustrated
by figs. 3-A and 4-A.   The data as to relative ash content of shoots grown
in these alcohols are shown in table III, and the molecular concentrations
of alcohol solutions required to produce equal quantities of ash in shoots
is shown in table IV.

TABLE III

Growth in solutions of alcohols and in calcium nitrate as indicated by relative ash content of shoots

Molecular concentration	Average of individual tests of 50 seedlings each											
	Methyl	Ethyl	N propyl	Iso-propyl	N butyl	Isobutyl	Secondary butyl	Tertiary butyl	N amyl	Isoamyl	N hexyl	Calcium nitrate
0.0012	51	67	52	53	56	53	53	53	48	63	35	79
0.0030	49	65	39	57	44	50	47	47	41	60	31	78
0.0090	47	64	23	56	38	39	53	51	32	53	19	76
0.0180	47	58	19	58	33	31	47	50	22	35	14	65
0.0300	47	54	17	52	24	21	56	50	15	26	1	64
0.0510	44	38	12	52	20	22	37	50	10	12	2	62
0.0600	47, 50	37, 24	12, 14	56	11, 7	19	50	52	6, 4	7	1	56

FIG. 4. Ash of soy-bean shoots grown in simple solutions of eight different alcohols.
Ordinates represent percentages of ash for standard solution.
Abscissas represent volume-molecular concentrations.

Growth in mixtures of alcohol and calcium nitrate

It has been found that mixtures of certain salts have an ameliorating effect with respect to toxicity as compared with growth of seedlings in solutions when each of the salts is used singly. The ion that exerts the most antagonistic effect seems to be calcium.

As previously stated, the author has attempted to determine the effect of a calcium salt on the growth of seedlings at different concentrations when the salt was mixed with certain normal alcohols—methyl, ethyl, propyl, butyl, and amyl.

TABLE IV

MOLECULAR CONCENTRATIONS OF SINGLE ALCOHOL SOLUTIONS REQUIRED TO PRODUCE EQUAL QUANTITIES OF ASH IN SHOOTS OF SEEDLINGS

RELATIVE GROWTH RATE	METHYL	ETHYL	N PROPYL	ISOPROPYL	N BUTYL	ISOBUTYL	SECONDARY BUTYL	TERTIARY BUTYL	N AMYL	ISOAMYL	N HEXYL
60	—	0.0006				—				0.0012	
50	0.0024	0.0354	0.0018	—	0.0018	0.003	0.0024	0.0024	0.0006	0.0102	0.0012
40		0.0486	0.003		0.0066	0.0084		—	0.0036	0.0156	0.0036
30		0.06	0.0066		0.0216	0.0192		—	0.0108	0.0246	0.0036
20	—		0.0162		0.051	0.0564	—		0.0210	0.0390	0.009
10			0.051		0.06				0.051	0.0546	.00216

In all instances but one, the toxic effect of these mixtures of alcohol and $Ca(NO_3)_2$ is greater than that of $Ca(NO_3)_2$ when used alone, or of the alcohol when used alone. The one exception was methyl alcohol and $Ca(NO_3)_2$. At concentrations approaching a total concentration of 0.03 M. there was evidence of antagonism as indicated by relative root elóngation.

It will be observed from fig. 5 that the increased toxicity due to mixtures is much greater when one uses a very toxic alcohol, such as propyl or amyl. In case of these alcohols the general outline of the mixture curves resembles the curve of the alcohol used singly.

LOEW (**16**) stated that it was necessary for certain metal ions to combine in a definite ratio with the proteins of the plant in order to produce normal growth. It seems that in this instance there was no combination of alcohols and protoplasm, or, if there was, the resultant combination was inimical to proper growth. The alcohols seem also to have prevented $Ca(NO_3)_2$ from becoming inert; or, by preventing it from being adsorbed, subsequently prevented adsorption by the plasma membrane.

METHYL ALCOHOL AND CALCIUM NITRATE.—Observation of the growth allowed by mixtures of methyl alcohol and $Ca(NO_3)_2$ (fig. 5) reveals an example of antagonism. The root elongation in mixtures is higher than in solutions where $Ca(NO_3)_2$ was used alone, and in part greater than when methyl alcohol was used singly. However, high salt content and low alcohol content, or low salt content and high alcohol content of solutions afforded a medium for growth more favorable than the mixture. Only where the components of the mixture approach equality is there evidence of antagonism.

ETHYL ALCOHOL AND CALCIUM NITRATE.—By referring to table V it will be found that in the mixtures of ethyl alcohol and $Ca(NO_3)_2$, where the components were present in equal molecular quantities, the root elongation was slightly less than the root elongation when the same amount of $Ca(NO_3)_2$ was used alone. However, the root elongation when equal quantities of the components were employed was 22 per cent. less than the root elongation, when ethyl alcohol alone was used in the same concentration as was used in the mixture.

It seems that the root elongation in mixtures containing high proportions of $Ca(NO_3)_2$ and small proportions of ethyl alcohol was not much different from the root elongation in solutions containing high proportions of ethyl alcohol and small proportions of $Ca(NO_3)_2$.

NORMAL PROPYL ALCOHOL AND CALCIUM NITRATE.—From fig. 5, it is evident that throughout all concentrations there is a marked increase of toxicity as indicated by root elongation when the mixtures of propyl alcohol and $Ca(NO_3)_2$ are used. At no point on the graphs do the lines intersect. The growth in a 2 per cent. solution of 0.06 M. $Ca(NO_3)_2$ is 100 per cent. better than in a solution containing a mixture of 2 per cent. solution of 0.06 M. $Ca(NO_3)_2$ and 98 per cent. of 0.06 M. normal propyl alcohol; and
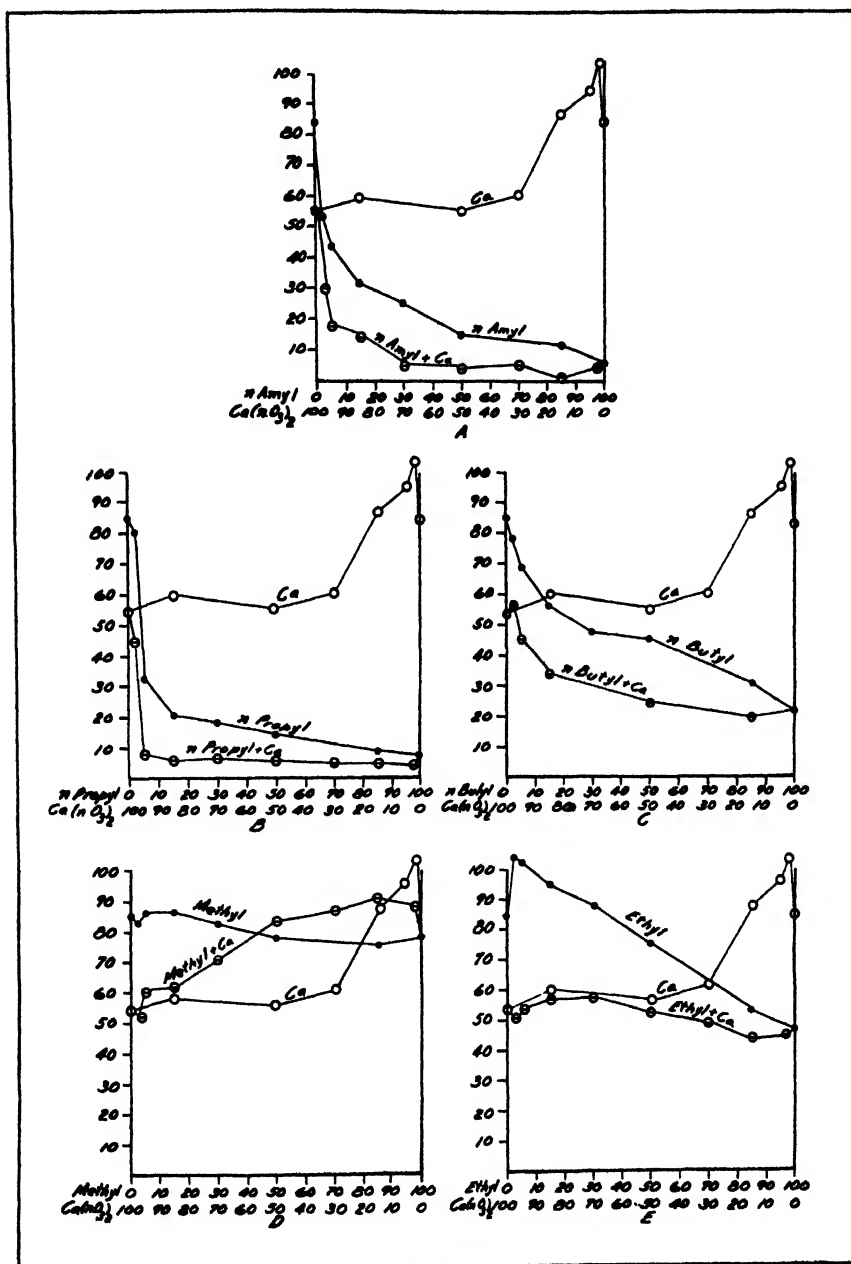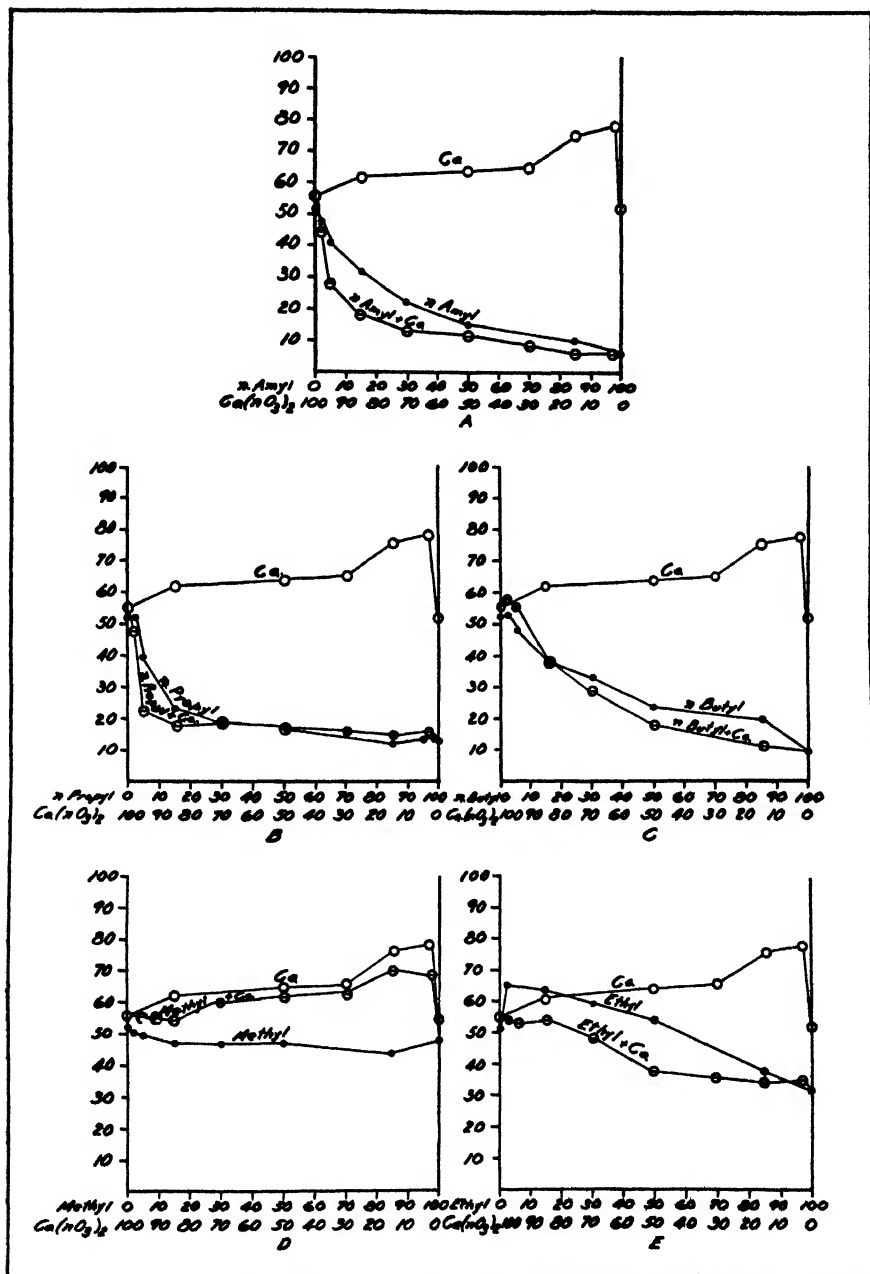
FIG. 5.   Elongation of soy-bean roots in mixed solutions of alcohol and calcium nitrate, and in simple solutions of these alcohols and calcium nitrate in the concentrations in which they exist in the mixed solutions.   Ordinates represent percentages of elongation for standard solution; abscissas represent molecular proportions.

TABLE V

GROWTH IN MIXED SOLUTIONS OF ALCOHOL AND CALCIUM NITRATE AS INDICATED BY ROOT ELONGATION; GROWTH VALUES REPRESENT THE AVERAGE OF FIFTY SEEDLINGS AND ARE EXPRESSED AS PERCENTAGES OF GROWTH FOR THE THREE-SALT STANDARD SOLUTION

Proportions of 0.06 M.		Methyl	Ethyl	Normal Propyl	Normal Butyl	Normal Amyl
$Ca(NO_3)_2$	Alcohol					
100	0	54	54	54	54	54
98	2	52	51	45	57	30
95	5	60	53	8	45	18
85	15	61	57	6	34	14
70	30	71	58	7	30	5
50	50	81	53	6	25	4
30	70	85	49	5		5
15	85	91	44	5	20	1
2	98	89	46	4		4
0	100	78	48	7	22	6

GROWTH IN MIXED SOLUTIONS OF ALCOHOL AND CALCIUM NITRATE AS INDICATED BY RELATIVE ASH CONTENT OF SHOOTS; GROWTH VALUES REPRESENT THE AVERAGE OF FIFTY SEEDLINGS, AND ARE EXPRESSED IN PERCENTAGE OF ASH CONTENT OF SHOOTS OF SEEDLINGS GROWN IN THE THREE-SALT STANDARD SOLUTION

Proportions of 0.06 M.		Methyl	Ethyl	Normal Propyl	Normal Butyl	Normal Amyl
$Ca(NO_3)_2$	Alcohol					
100	0	55	55	55	55	55
98	2	55	54	48	65	44
95	5	53	53	22	47	28
85	15	53	54	18	38	18
70	30	60	45	19	29	13
50	50	62	37	17	18	12
30	70	64	35	15		8
15	85	70	34	14	11	6
2	98	70	34	14		6
0	100	49	31	13	9	5

FIG. 6. Ash of soy-bean shoots grown in mixed solutions of alcohol and calcium nitrate, and in simple solutions of these alcohols and calcium nitrate in the concentrations in which they exist in mixed solutions. Ordinates represent percentages of ash for standard solution; abscissas represent molecular proportions.

a solution containing 2 per cent. of 0.06 M. normal propyl alcohol and 98 per cent. of 0.06 M. Ca(NO₃)₂ allows a growth that is 36 per cent. less than the growth in a solution containing 2 per cent.of 0.06 M. propyl alcohol singly.

NORMAL BUTYL ALCOHOL AND CALCIUM NITRATE.—The toxicity of mixtures of normal butyl alcohol and Ca(NO₃)₂ is found to be greater than when each of the components was used singly. This increased toxicity is not so pronounced as in the case of normal propyl alcohol, yet it is obvious as seen in fig. 5.

A solution containing the components 98 per cent. of 0.06 M. Ca(NO₃)₂ and 2 per cent. of 0.06 M. normal butyl alcohol allows a growth rate 22 per cent. less than does a solution containing 2 per cent. normal butyl alcohol used singly. The components 15 per cent. of 0.06 M. Ca(NO₃)₂ and 85 per cent. of 0.06 M. normal butyl alcohol allows a growth rate that is 68 per cent. less than the growth in a solution 15 per cent. of 0.06 M. Ca(NO₃)₂ solution used singly.

NORMAL AMYL ALCOHOL AND CALCIUM NITRATE.—Normal amyl, one of the most toxic alcohols used, when mixed with Ca(NO₃)₂ exerts a marked increase of toxicity as compared to the growth when each of the components was used singly. As the toxicity of normal amyl alcohol resembles the toxicity of normal propyl alcohol in some respects, so the increased toxicity due to mixing normal amyl alcohol and Ca(NO₃)₂ is comparable to the increased toxicity of mixtures of normal propyl alcohol and Ca(NO₃)₂ over solutions of the components used singly.

Throughout all concentrations this increased toxic effect prevails. The growth in a solution containing a mixture of 2 per cent. of 0.06 M. amyl alcohol and 98 per cent. of 0.06 M. Ca(NO₃)₂ is about 24 per cent. less than the growth in a single solution containing 2 per cent. of 0.06 M. amyl alcohol.

The growth allowed in a solution containing a mixture of 98 per cent. of 0.06 M. normal amyl alcohol and 2 per cent. of 0.06 M. Ca(NO₃)₂ was 100 per cent. less than in a single salt solution containing 2 per cent. of 0.06 M. solution of Ca(NO₃)₂.

The growth in a solution containing a mixture of Ca(NO₃)₂ and amyl alcohol was approximately the same as the growth in a single solution of hexyl alcohol, comparing solutions having equal molar quantities of alcohol.

COMPARISONS OF TWO CRITERIA OF GROWTH IN MIXTURES.—The difference between the growth in single alcohol solution and the growth in solutions containing alcohol and Ca(NO₃)₂ is usually less when the relative ash content is used as a criterion than when the root elongation is used.

There are some individual differences also which are noticeable. The degree of antagonism—increased growth in solutions of mixtures over that

in single solutions—is shown to be numerically higher when the criterion of ash weight is used as a basis of growth measurement. This feature seems evident when the difference in growth between that in single methyl alcohol solutions and growth in mixtures of methyl alcohol and $Ca(NO_3)_2$ is noted. On the other hand, when the ash criterion is used, the difference in growth in a single solution of $Ca(NO_3)_2$ and the growth in a solution containing a mixture of $Ca(NO_3)_2$ and methyl alcohol indicates an increased toxic effect. But when the rate of root elongation is used as a criterion the data indicates for the same instance, antagonism.

Another example where the two methods of measuring growth seem to vary is the case of normal butyl alcohol. The curves (fig. 6) indicate that there is little difference between the growth of seedlings in normal butyl alcohol used singly, and in mixtures of normal butyl alcohol and $Ca(NO_3)_2$. On the other hand, when the root elongation is the criterion (fig. 5) the growth rate appears to be considerably higher in the case of single solutions than in the corresponding mixture.

Summary

A study was made of elongation of soy-bean roots and the ash content of the shoots of these plants with simple solutions of calcium nitrate and the following alcohols: methyl, ethyl, normal propyl, isopropyl, normal butyl, isobutyl, secondary butyl, tertiary butyl, normal amyl, isoamyl, and normal hexyl. The single solutions varied in concentration from 0.0012 M. to 0.06 M. With each total concentration nine different sets of alcohol proportions were tested. Soy-bean seedlings having roots with an initial length of about 8 mm. were placed in the culture solutions and grown in darkness. Each test was terminated when the roots in a standard solution had attained a length of 95 mm. Amounts of root elongation, expressed as percentages of the elongation in the standard solution were used as the basis for quantitative comparisons of the physiological effects of the culture solutions. Amounts of ash of shoots, expressed as percentages of the ash of shoots in standard solutions were also used. The principal results and conclusions follow:

1. With the exception of the less toxic alcohols, the root elongation and ash content of shoots were lower in single alcohol solutions than in distilled water. Elongation was inversely related to concentration, but no simple rule applicable to every case can be formulated regarding the relationship of toxicity to concentration over the range of concentrations tested.

2. Single solutions of ethyl alcohol and of calcium nitrate at low concentrations exert a stimulating influence for increasing growth. At a concentration of 0.0012 M. these afford a medium for growth better than that of a standard solution. This stimulating influence is less pronounced in the case of isobutyl and isopropyl alcohol.

3. When root elongation is the criterion for growth, the order of toxicity (from least to most toxic) for moderate retardation (0.003 M.) is as follows: isopropyl, methyl, ethyl, secondary butyl, calcium nitrate, normal butyl, isobutyl, tertiary butyl, normal amyl, normal propyl, isoamyl, and normal hexyl. For high retardation (0.06 M.) the order is as follows: methyl, isopropyl, secondary butyl, calcium nitrate, ethyl, normal butyl, isobutyl, normal propyl, tertiary butyl, normal amyl, isoamyl, and normal hexyl.

4. When relative ash content is used as a criterion, the order of toxicity (from least to most toxic) for moderate retardation (0.03 M.) is as follows: calcium nitrate, secondary butyl, ethyl, isopropyl, tertiary butyl, methyl, isoamyl, normal butyl, isobutyl, normal propyl, normal amyl, normal hexyl. At high retardation (0.06 M.) the order is as follows: isopropyl, calcium nitrate, tertiary butyl, secondary butyl, methyl, ethyl, isobutyl, normal propyl, normal butyl, isoamyl, amyl, normal hexyl. Relatively more ash was found in shoots grown in a solution of 0.0012 M. $Ca(NO_3)_2$ than in those grown in solutions of higher concentration.

5. When seedlings were grown in mixtures of calcium nitrate and methyl alcohol there was evidence of slight antagonism—the solutions were not quite as toxic as when the components were used singly. When the individual alcohols, ethyl, normal propyl, normal butyl, and normal amyl were used in mixtures with calcium nitrate, the toxic effects were increased—the toxic effect of the mixtures was greater than the toxic effect when the components were used singly. This increase was most pronounced when normal amyl was used in mixtures with calcium nitrate.

6. The toxicity of alcohols does not seem to increase regularly with increased number of carbon atoms in the molecules. Not in all instances is the normal alcohol more toxic than the iso-alcohol.

7. Equations are given for computing approximate growths.

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LITERATURE CITED

1. BIRNER, H., and LUCANUS, B. Wasserculturversuche mit Hafer. Landw. Versuchs-St. 8:128–177. 1866.

2. CHICK, H. An investigation of the laws of disinfection. Jour. Hygiene 8: 92–158. 1908.

3. DANIEL, J. F. Adaptation and immunity of lower organisms to ethyl alcohol. Jour. Exp. Zool. 6: 572–611. 1909.

4. EISENMENGER, W. S. Toxicity, additive effects, and antagonism of salt solutions as indicated by growth of wheat roots. Bull. Torrey Bot. Club 55: 261–304. 1928.

5. FALK, I. S., and WINSLOW, C.-E. A.   A contribution to the dynamics of toxicity and the theory of disinfection.   Jour. Bact. **11**: 1–25. 1926.

6. HODGE, C. F.   Experiments on the physiology of alcohol made under the auspices of the committee of fifty.   Pop. Science Monthly **60**: 594–603. 1897.

7. JOHNSTON, E. S., and DORE, W. H.   The relation of boron to the growth of the tomato plant.   Science n.s. **67**: 324–325. 1928.

8. KNOP, W.   Ein Vegetationsversuch.   Landw. Versuchs-St. **1**:181–202. 1859.

9. —————.   Quantitativ-analytische Arbeiten über den Ernähr-ungs-process der Pflanzen.   Landw. Versuchs-St. **4**: 173–187. 1862.

10. KRONIG, B., and PAUL, T.   Die chemischen Grundlagen der Lehre von der Giftwirkung und Desinfection.   Zeitschr. Hygiene und Infectionskrankheiten **25**: 1–112. 1897.

11. LeRENARD, A.   Influence du milieu sur la résistance du Pénicille crustacé aux substances toxiques.   Ann. Sci. Nat. Bot. IX. **16**: 277–336. 1912.

12. LOEB, J.   On ion-protein compounds and their rôle in the mechanics of life phenomena. I. The poisonous character of a pure NaCl solution.   Amer. Jour. Physiol. **3**: 327–338. 1900.

13. —————.   Ueber den Einfluss der Wertigkeit und möglicher Weise der electrichen Ladung von Ionen auf ihre antitoxische Wirkung.   Archiv Ges. Physiol. **88**: 68–78. 1902.

14. —————.   The dynamics of living matter.   New York.   1906.

15. —————.   The mechanistic conception of life.   Chicago.   1912.

16. LOEW, O.   Ueber die physiologischen Functionen der Calcium- und Magnesium-salze im Pflanzenorganismus.   Flora **75**: 368–394. 1892.

17. MALTAUX, MARIE, and MASSART, JEAN.   Sur les excitants de la division cellulaire.   Ann. Soc. roy. sci. méd. et nat. de Bruxelles, **15**:fasc. 3, 1–53.   Recueil de l'institute Botanique. **6**: 369–421. 1906.

18. McCOLLUM, E. V.   Organic chemistry for students of medicine and biology.   New York.   1917.

19. McHARGUE, J. S.   Effect of different concentrations of manganese sulphate on the growth of plants in acid and neutral soils and the necessity of manganese as a plant nutrient.   Jour. Agr. Res. **24**: 781–794. 1923.

20. MUNCH, J. C., and SCHWARTZE, E. W.   Narcotic and toxic potency of aliphatic alcohols upon rabbits.   Jour. Lab. and Clin. Med. **10**: 985–996. 1925.

21. OSTERHOUT, W. J. V.   On the importance of physiologically balanced solutions for plants. II.   Fresh water and terrestrial plants.   Bot. Gaz. **44**: 259–272. 1907.

22. ——————.   Quantitative criteria of antagonism.   Bot. Gaz. **58**: 178–186. 1914.
    The measurement of antagonism.   Bot. Gaz. **58**: 272–276. 1914.

23. ——————.   The determination of additive effects.   Bot. Gaz. **60**: 228–234. 1915.

24. ——————.   The basis of measurement of antagonism.   Jour. Biol. Chem. **34**: 363–368. 1918.

25. PAUL, T., BIRSTEIN, G., and REUSS, A.   Beiträge zur Kinetik der Giftwirkung von gelösten Stoffen.   Biochem. Zeitschr. **29**: 202–278. 1910.

26. RAUBER, A.   Wirkung des Alkohols auf Tiere und Pflanzen.   Leipzig. 1902.

27. RICHARDSON, B. W.   Our common alcohol and alcohol family.   p. 23. London. 1883.

28. ——————.   Lectures on experimental and practical medicine. Physiological research on alcohols.   Med. Times and Gaz. **2**: 703–706. 1869.

29. SHIVE, J. W.   A study of physiological balance in nutrient media. Physiol. Res. **1**: 327–397. 1915.

30. SOMMER, A. L.   The search for elements essential in only small amounts for plant growth.   Science n.s. **66**: 482–484. 1927.

31. ——————, and LIPMAN, C. B.   Evidence on the indispensable nature of zinc and boron for higher green plants.   Plant Physiol. **1**: 231–249. 1926.

32. TOTTINGHAM, W. E.   A quantitative chemical and physiological study of nutrient solutions for plant cultures.   Physiol. Res. **1**: 133–245. 1914.

33. TRELEASE, S. F., and FREE, E. E.   The effect of the renewal of culture solutions on the growth of young wheat plants in water culture. Johns Hopkins Univ. Circ. **3**: 227–228. 1917.

34. ——————, and LIVINGSTON, B. E.   Continuous renewal of nutrient solution for plants in water cultures.   Science n.s. **55**: 483–486. 1922.

35. ——————, and TRELEASE, H. M.   Relation of seed weight to growth and variability of wheat in water cultures.   Bot. Gaz. **77**: 199–211. 1924.

36. ——————, ——————.   Growth of wheat roots in salt solutions containing essential ions.   Bot. Gaz. **80**: 74–83. 1925.

CHANGES IN THE COMPOSITION OF THE TOMATO PLANT ACCOMPANYING DIFFERENT STAGES OF YELLOWS[1,2]

Michael Shapovalov and Henry A. Jones

Purpose of this investigation

In 1927 Rosa[3] published data which show that tomato yellows is characterized by definite changes in chemical composition in all parts of the host plant. His material was collected from healthy as well as from the affected plants showing different degrees of severity of the disease. Not only were the amounts of certain constituents in healthy plants different from those in blighted plants, but there was also a considerable variation in the composition of the different diseased samples. However, it was not apparent that this variation was definitely associated with different stages of the disease, since the samples obtained from different plants and on different days were not strictly comparable. Furthermore, the identification of the disease in the case of Rosa's material was based exclusively on external appearances of the plants used. There was no knowledge of either the previous history or the etiology of these abnormal appearances. It was not certain that the results obtained with this material could likewise be applied to the seemingly identical pathological phenomenon induced by the curly-top virus through the medium of the beet leafhopper, *Eutettix tenellus* Baker.

It still remained to be shown, therefore, whether the peculiar chemical changes observed in connection with tomato yellows accumulate in the plant gradually as the diseased conditions become more and more severe, and whether the field phenomenon known as tomato yellows is identical in its inner processes as well as in its extrinsic symptomatology with the disease caused by the curlytop virus. To show this, it was necessary to use artificially inoculated material and to analyze different stages of the disease as represented by different plants on the same day, as well as various stages of yellows shown by the same plant on different dates. In addition to this, it appeared desirable to ascertain whether there is a difference in the response to the yellows infection between the known resistant and the susceptible varieties. For this purpose several resistant strains developed

[3] Rosa, J. T. Chemical changes accompanying the western yellow blight of tomato. Plant Physiol. 2: 163–169. 1927.

by Dr. J. W. LESLEY were used. It was Dr. ROSA's intention to continue the work along the lines indicated above, but these plans were left unfulfilled because of his untimely death. The writers considered it very urgent to complete this phase of the work. The inoculations were made and the samples preserved at the Citrus Experiment Station, Riverside, California; the analyses were made in the division of Truck Crops, Davis, California.[4]

Methods employed

To obtain the diseased samples for analytical purposes a number of plants were inoculated by allowing viruliferous beet leafhoppers to feed on these plants. The following method of inoculation was followed: A celluloid cell, about 5 inches long and 2.5 inches in diameter, with muslin ends, was placed over the tip of each plant, and several insects injected into each cell. The number of insects used in different series of inoculations varied from 5 to 20, but each plant in a given series received the same number. Likewise the period during which the insects were allowed to feed on the plant varied in different series from 2 to 6 days, but it was the same for every plant in a given series. One series was inoculated in November, 1927 (table I), one in the spring of 1928 (table II) and one in the summer of 1928 (table III). When possible, samples were taken at different intervals from the same inoculated plants, as well as from the same healthy plants used for checks.

The plants used can be identified by their numbers in each series. In the first series inoculated plants nos. 2 and 3 were omitted and a new plant no. 5 was introduced, for the reason that the former did not develop the disease while the latter did. Plant no. 1 in the second series was used in its entirety during the first sampling on June 16. No substitution for the check plants was made in any of the series. Samples in series no. 3 (table III) were taken in Dr. J. W. LESLEY's field. The following types of material were selected: (a) Uninoculated healthy plants; (b) plants inoculated, but showing no symptoms of yellows; (c) plants showing an early or medium stage of yellows, represented by a complex of symptoms preceding distinct yellowing, and (d) plants showing a late stage of yellows, when the characteristic sulphur-yellow discoloration is well marked. Detailed descriptions of these samples and the results of the analyses are given in Tables I, II, and III.

Fifty-gram samples were collected shortly before noon. They were immediately preserved in boiling 95 per cent. alcohol. After drying the solid portion of the sample, and grinding, extraction was completed with 50 per cent. alcohol. Carbohydrates were determined by the method used

TABLE I

Composition of inoculated tomato plants in different stages of the development of yellows in comparison with uninoculated healthy checks. Series 1, inoculated November 9, 1927. All plants except checks were inoculated. Results expressed in percentage of fresh weight

Plant number	Date of collecting samples	Condition of material	Dry matter	Dex-trose	Su-crose	Starch	Nitrogen			
							Soluble	Insoluble	Total	Per cent. soluble
		Leaves								
1	11-17-27	Check, healthy[a]	10.0	0.16	0.00	0.17	0.034	0.286	0.320	10.7
	12-12-27	Check, healthy[a]	10.8	0.25	0.18	0.41	0.074	0.244	0.318	23.2
	1-18-28	Check, healthy[a]	13.3	0.31	0.38	0.59	0.049	0.438	0.487	10.0
2	11-17-27	Apparently healthy	11.6	0.18	0.22	0.20	0.124	0.237	0.361	34.4
3	11-17-27	Apparently healthy	14.3	0.24	0.24	0.98	0.063	0.453	0.516	12.3
4	11-17-27	Apparently healthy	14.8	0.24	0.23	1.04	0.073	0.374	0.447	16.3
	12-12-27	Early medium stage of yellows[b]	16.9	0.61	0.34	1.79	0.071	0.424	0.495	14.3
	1-18-28	Late stage of yellows	16.9	1.42	0.53	1.74	0.071	0.329	0.400	17.7
5	12-12-27	Early medium stage of yellows	16.5	0.68	0.40	1.68	0.063	0.373	0.436	14.5
	1-18-28	Late stage of yellows	16.9	1.33	0.63	1.15	0.102	0.367	0.469	21.8
		Stems								
1	11-17-27	Check, healthy[a]	8.6	0.57	0.16	0.05	0.178	0.120	0.298	59.6
	12-12-27	Check, healthy[a]	8.7	0.68	0.24	0.16	0.090	0.112	0.202	44.6
	1-18-28	Check, healthy[a]	8.7	0.39	0.29	0.16	0.095	0.127	0.222	42.8
2	11-17-27	Apparently healthy	7.7	0.36	0.13	0.05	0.151	0.120	0.271	55.7
3	11-17-27	Apparently healthy	7.4	0.48	0.17	1.01	0.114	0.101	0.215	53.1
4	11-17-27	Apparently healthy	8.4	0.60	0.19	0.20	0.112	0.110	0.222	50.4
	12-12-27	Early medium stage of yellows[b]	13.6	1.54	0.79	0.69	0.041	0.139	0.180	22.9
	1-18-28	Late stage of yellows	15.8	1.17	1.15	1.98	0.002	0.186	0.188	1.1
5	12-12-27	Early medium stage of yellows	13.6	1.42	0.89	1.03	0.066	0.141	0.207	31.9
	1-18-28	Late stage of yellows	12.3	1.30	1.09	1.32	0.001	0.122	0.123	0.9

[a] Same check plant.   [b] Only plant number 4 showed symptoms of the disease, while plants 2 and 3 did not, and were discarded.

TABLE II

Composition of inoculated tomato plants in different stages of the development of yellows in comparison with uninoculated healthy checks. Series 2, inoculated June 28, 1928. All plants except checks were inoculated. Results expressed as percentage of fresh weight

Plant numbers	Date of collecting samples	Condition of material	Dry matter	Dextrose	Sucrose	Starch	Nitrogen			
							Soluble	Insoluble	Total	Per cent soluble
		Leaves								
		A								
1*	7-16-28	Yellows in advanced stage, very chlorotic	17.3	1.29	0.57	3.43	0.092	0.295	0.387	23.9
2	7-16-28	Yellows in early stage, leaves rigid, but not very chlorotic	15.8	0.57	0.30	3.23	0.029	0.127	0.156	18.6
	7-30-28	Advanced stage of yellows	16.3	1.37	0.34	4.47	0.017	0.579	0.596	2.9
3	7-16-28	Similar to no. 2 on this date, but a little more advanced; slightly more discoloration	17.7	0.54	0.29	3.44	0.063	0.339	0.402	15.7
	7-30-28	Slightly more advanced than no. 2 on this date	17.6	1.22	0.59	3.53	0.001	0.558	0.559	0.2
4	7-16-28	Apparently healthy	12.9	0.30	0.26	1.31	0.068	0.423	0.491	13.8
	7-30-28	Still healthy	13.3	0.25	0.31	0.68	0.007	0.389	0.396	1.8
5	7-16-28	Apparently healthy	12.4	0.26	0.17	1.11	0.049	0.321	0.370	13.2
	7-30-28	Still healthy	12.7	0.18	0.34	1.31	0.056	0.405	0.461	12.1
6	7-16-28	Check. Healthy	11.8	0.30	0.25	0.52	0.074	0.384	0.458	16.1
	7-30-28	Check. Healthy	12.0	0.36	0.22	1.69	0.063	0.446	0.520	12.4
		B								
9*	7-30-28	Check. Healthy	13.0	0.38	0.35	1.27	0.054	0.375	0.429	12.5
10	7-30-28	Check. Healthy	12.6	0.31	0.37	1.08	0.056	0.375	0.431	13.0
8	7-30-28	Between medium and advanced stage of yellows	15.2	0.57	0.27	1.89	0.073	0.289	0.362	20.2
7	7-30-28	Advanced stage of yellows	17.3	0.83	0.29	3.66	0.056	0.283	0.339	16.5

* Numbers 1 to 6 in this table group A are the Stone variety; 7 to 10 group B, resistant selections, no. 0.012 of Dr. J. W. Lesley.

TABLE II—Continued

PLANT NUMBER	DATE OF COLLECTING SAMPLES	CONDITION OF MATERIAL	DRY MATTER	DEXTROSE	SUCROSE	STARCH	NITROGEN SOLUBLE	NITROGEN INSOLUBLE	NITROGEN TOTAL	PER CENT. SOLUBLE
		Stems								
		A								
1*	7-16-28	Yellows in advanced stage	13.5	1.44	1.31	0.54	0.152	0.159	0.311	48.9
2	7-16-28	Yellows in early stage, leaves rigid, but not very chlorotic	11.2	1.30	0.69	0.65	0.080	0.129	0.209	38.3
	7-30-28	Advanced stage of yellows	13.0	118	1.29	1.23		0.143		—
3	7-16-28	Similar to no. 2 on this date but a little more advanced; slightly more discoloration	10.9	0.98	0.55	0.52	0.092	0.125	0.217	42.5
	7-30-28	Slightly more advanced stage of yellows than no. 2 on this date	14.5	0.99	1.33	0.79	0.156	0.157	0.313	49.8
4	7-16-28	Apparently healthy	7.5	0.55	116	0.16	0.044	0.083	0.127	34.5
	7-30-28	Still healthy	9.4	0.55	0.26	0.25	0.054	0.098	0.152	35.5
5	7-16-28	Apparently healthy	8.3	0.38	0.17	0.16	0.083	0.094	0.177	46.8
	7-30-28	Still healthy	8.7	0.60	0.21	0.15	0.080	0.100	0.180	44.5
6	7-16-28	Check. Healthy	8.4	0.45	0.16	0.20	0.092	0.112	0.204	45.2
	7-30-38	Check. Healthy	8.3	0.73	0.33	0.18	0.102	0.086	0.188	54.3
		B								
9*	7-30-28	Check. Healthy	10.6	0.70	0.38	0.13	0.051	0.097	0.148	34.4
10	7-30-28	Check. Healthy	9.4	0.63	0.25	0.16	0.075	0.097	0.172	43.7
8	7-30-28	Between medium and advanced stage of yellows	13.6	0.83	0.91	0.61	0.139	0.155	0.294	47.3
7	7-30-28	Advanced stage of yellows	14.8	0.84	0.97	0.95	0.119	0.137	0.256	46.6

* Numbers 1 to 6 in this table group A are the Stone variety; 7 to 10 group B, resistant selections, no. 0.012 of Dr. J. W. LESLEY.

by BISSON and SEWELL[5]—sucrose after inversion with invertase ($\frac{1}{3}$ cc. in 100 for 24 hours), and starch after autoclaving for one hour and digesting with a buffered solution of taka-diastase (pH = 4.66) for 24 hours. A blank was run using soluble starch, and the factor from that recovery was used to calculate the results of the other starches. Total nitrogen was determined by the official Gunning method. Nitrogen was determined separately on aliquots of the insoluble and alcohol-soluble portions of each sample.

Results of the analyses

The results obtained with artificially inoculated tomato plants are in general accord with the data published by ROSA. The composite samples listed in table III gave the closest parallel to ROSA's findings which likewise were based on composite samples. The behavior of individual plants, however, shows considerable variation, although the main process in the affected plants appears to be the same as indicated by composite samples.

Dry matter.—The percentage of dry matter always appeared to be greater in both leaves and stems of the plants inoculated with viruliferous beet leafhoppers, after the infected plants developed external symptoms of yellows, than in those of healthy checks. This is true of the tested resistant selections as well as of the susceptible variety, Stone. When inoculated plants failed to develop visible symptoms of the disease the amount of dry matter was about the same as in corresponding checks, as shown by plants 4 and 5 in table II. Advanced stages of yellows, whether in one and the same plant or in different plants, in the case of susceptible as well as resistant strains, were accompanied by larger amounts of dry matter than medium or initial stages. An exception is presented by leaves of plants 2 and 3 in table II, which showed practically unchanged percentage of dry matter at two different samplings made two weeks apart, although in both cases higher than in checks on same dates. These plants also revealed an increase in total and insoluble nitrogen and a decrease of its soluble portion. During the winter months (series 1) the percentage of dry matter increased in the healthy check plants also, although this increase appeared to be limited to the leaves and was not apparent in the stems.

Carbohydrates.—Leaves and stems of the inoculated plants of all the tested strains showed decidedly higher carbohydrate contents than in the case of uninoculated checks on the same dates. The results obtained with composite samples were again most strikingly consistent, especially with stems of these samples. Plants showing advanced symptoms of yellows, as a rule appeared to be more abundantly supplied with carbohydrates than those which were still in earlier stages of the disease, although there were

[5] BISSON, C. S., and SEWELL, J. GORDON. The estimation of cuprous oxide produced in sugar analysis. Jour. Assoc. Offic. Agr. Chem. 10: 120–124. 1927.

TABLE III

COMPOSITION OF YELLOWS RESISTANT AND YELLOWS SUSCEPTIBLE TOMATO PLANTS. ALL SAMPLES IN THIS SERIES (3) ARE COMPOSITE, INOCULATED AND DISEASED AS WELL AS UNINOCULATED HEALTHY, COLLECTED ON SEPTEMBER 5, 1928. RESULTS EXPRESSED AS PERCENTAGE OF FRESH WEIGHT

SAMPLE NUMBER	CONDITION OF MATERIAL	DRY MATTER	DEX-TROSE	SU-CROSE	STARCH	NITROGEN			
						SOL-UBLE	INSOL-UBLE	TOTAL	PER CENT. SOLUBLE
	Leaves								
1*	Healthy dwarf (resistant) no. .006	15.2	0.37	0.30	2.04	0.039	0.313	0.352	11.1
2	Inoculated and diseased " "	18.1	1.58	0.62	2.81	0.061	0.233	0.294	20.7
3	Healthy standard (resistant) no. .018	15.4	0.34	0.20	1.25	0.039	0.260	0.299	13.0
4	Inoculated and diseased " "	17.1	0.40	0.45	1.90	0.044	0.220	0.264	16.6
5	Healthy standard (susceptible) no. .014				2.05	0.039	0.277	0.316	12.3
6	Inoculated and diseased " "				3.86		0.221		
	Stems								
1*	Healthy dwarf (resistant) no. .006	14.2	1.01	1.46	0.56	0.032	0.089	0.121	26.4
2	Inoculated and diseased " "	19.0	1.48	2.61	2.67	0.092	0.134	0.226	40.7
3	Healthy standard (resistant) no. .006	14.7	1.00	0.53	0.59	0.054	0.091	0.145	37.2
4	Inoculated and diseased (resistant) no. .018	17.7	1.47	1.83	1.46	0.073	0.116	0.189	38.7
5	Healthy standard (susceptible) no. .014	17.4	0.97	1.26	1.28	0.034	0.105	0.139	24.5
6	Inoculated and diseased " "	20.6	1.14	2.25	2.63	0.074	0.097	0.171	43.2

* In numbers 1 and 2, a composite of 3 plants was used for a sample. In numbers 3 to 6, a composite of 4 plants was used.

a few insignificant exceptions.   While sugars as well as starch tend to accumulate in the inoculated and affected plants, this process is particularly pronounced and constant in the case of starch.

Nitrogen.—Changes in the nitrogen contents in connection with the development of yellows in artificially inoculated plants were somewhat variable although the general direction in which these changes proceed may still be recognized.   There was a tendency for total nitrogen to decrease in the affected leaves at the expense of the insoluble fraction, as is well shown by the composite samples of series 3 (table III) and by the resistant individual plants in series 2 (table II, B).   Stems of the same plants, on the contrary, showed a decided tendency for total nitrogen to increase.   The data obtained with leaves and stems of the affected susceptible plants are somewhat contradictory.   The same lack of complete uniformity may be observed with respect to the percentage of soluble nitrogen.   It is higher in the composite samples of series 3 and the resistant individuals of series 2, both in leaves and stems, but the susceptible variety, Stone, showed different tendencies in different plants with no distinct difference between the leaves and the stems.

Internal vs. external changes.—The composition of individual plants in different stages of the development of yellows shows very clearly that the accumulation of dry matter, starch and sugars, is progressive and gradual, and as a rule corresponds to the severity of the external symptoms of the disease.   With some plants this internal process is more marked in the leaves, with others, in the stems.

With respect to nitrogen, our results do not indicate any gradual or progressive changes which are consistent.   Plant 4 in series 1 showed a decrease in both total and insoluble nitrogen in leaves, and their increase in stems, whereas quite opposite changes seemed to take place in plant 5; the percentage of the soluble fraction increased in the leaves of both plants, while showing a tremendous decrease in the stems.   The predominating tendency in series 2 was more apparent than in series 1.   With the exception of sample 4, there was a decided increase in total nitrogen and its insoluble fraction in the leaves and stems.   Sample 4 showed a decrease of these constituents in the leaves and their increase in the stems, quite like plant 4 in series 1.   The most surprising phenomenon was the decrease in the percentage of soluble nitrogen in the leaves, particularly in the case of samples 2, 3 and 4.   With one exception, there was an increase of this fraction in the stems.

It is difficult to explain the striking decrease of soluble nitrogen in the leaves of plants 2, 3 (inoculated and diseased) and 4 (inoculated but not externally diseased) of series 2 on the basis of the present limited knowledge of internal changes that accompany the phenomenon known as tomato

yellows. It is possible that this decrease was due to the inactivation of virus, and the resumption of growth by the inoculated plants. This renewed growth activity may account for the decrease in the percentage of nitrogen in the soluble fraction.

Conclusions

1. Chemical changes accompanying external symptoms of tomato yellows produced by artificial inoculations with curlytop virus are essentially identical with those which accompany a similar complex of symptoms observed in the field and formerly known under the name of western yellow blight and other synonyms.

2. Among these changes the increase of dry matter and the accumulation of starch and sugars in the leaves as well as in the stems of the inoculated and naturally affected plants appeared to be constant and should be regarded as a condition characteristic of this virus malady.

3. Changes in the amounts of nitrogen are variable. There is often a decrease of total nitrogen, particularly in the leaves, at the expense of its insoluble fraction, with an increase in the percentage of the soluble portion. However, these changes are not constant and therefore may not be typical for tomato yellows.

4. The carbohydrate accumulation in the same plant is progressive, and larger amounts of these constituents are definitely connected with more severe external symptoms.

5. Resistant strains show essentially the same response to the yellows infection as do susceptible strains when composite samples taken on the same day are compared: A definite increase in the carbohydrate contents and an apparent tendency toward the increase in the soluble nitrogen and the decrease in the total and the insoluble portions in the leaves and *vice versa* in the stems, with some exception in the composite samples.

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THE FOURTH PACIFIC SCIENCE CONGRESS

E. M. HARVEY

(WITH SEVEN FIGURES)

The Fourth Pacific Science Congress was held in Batavia and Bandoeng, Java, May 16 to 25, 1929, under the auspices of the Netherlands Indies Science Council acting as a member of the Pacific Science Association. The Netherlands East Indies government cooperated with the Council in every way to assure the success of the Congress.

Overseas delegates were guests of the Netherlands East Indies government and Science Council, not only during the actual duration of the meetings, but also for all the numerous excursions provided from May 12 to June 5, throughout Java and neighboring islands. In his address which officially opened the meeting in Batavia, His Excellency, the Governor General of the Netherlands Indies, pleasantly expressed the hope that the American delegates would experience a "Dutch treat" in a sense otherwise than the one in which the term is often used "rather ungraciously to Dutch hospitality." It may be said with full assurance that His Excellency's hope was completely realized! Space will not permit so much as mention of the numerous interesting and sometimes highly instructive entertainments offered the delegates. Scarcely more than mention can be made of the principal excursions of interest to botanists and agriculturists. It may be added in passing, that the special scientific excursions were probably of equal, and sometimes of greater value than the meetings themselves. When one considers how full of treasures of scientific interest is that region of the world, it is not difficult to understand the enthusiasm of the delegates for studying nature in the East Indies first hand. The administration of the Congress anticipated this desire and provided for it most generously.

In attendance were 191 delegates (members) and perhaps 75 to 100 participants. Fig. 1* shows the group of delegates and participants and fig. 2, the Executive Committee of the Congress. Twenty-four countries were represented. Among the fifty or more delegates who may be classified as Botanists, a few will be mentioned as follows: Dr. D. VAN LEEUWEN, Director of the Botanical Gardens, Buitenzorg; Dr. F. VON FABER, Director of the Treub Laboratory, Buitenzorg; Dr. M. MYOSHI, Emeritus Professor of Botany, Tokyo Imperial University; Dr. F. A. F. C. WENT, Professor of Botany, University of Utrecht, and President of the Royal Academy of Sciences, Amsterdam; Dr. C. J. SKOTTSBERG, Director, Botanic Gardens, Gothenburg, Sweden; Dr. E. J. GODDARD, Dean of Agriculture,

* The figures are copied from illustrations furnished by the Congress.

University of Queensland, Brisbane; Dr. H. HOTTORI, Director, Tokugawa Biological Institute, Tokyo; Dr. W. A. SETCHELL, University of California; and Dr. F. V. COVILLE, U. S. Department of Agriculture.   These are but a few of the distinguished members of the Congress, and other equally distinguished technical agriculturists and biochemists were in attendance. At least one should mention the name of Dr. O. DE VRIES, Director of the Rubber Experiment Station, Buitenzorg, and President of the Science Congress.   Three members of the American Society of Plant Physiologists were present; namely, Dr. K. KORIBA, Professor of Botany, Kyoto Imperial University; Dr. G. A. C. HERKLOTS, University of Hong Kong; and the writer.

FIG. 1.   Delegates and participants to the Fourth Pacific Science Congress, Bandoeng, Java, May, 1929.

The meetings were opened officially in Batavia, May 16, but the scientific program did not commence until May 18 in Bandoeng, where the meetings continued to May 25.   Bandoeng was regarded as a more favorable location for the meetings than Batavia, on account of its higher altitude (2100 ft.) and consequent lower temperature.

One special feature of the program were the several symposia on topics, some of which were of general interest, and others of interest only to specific groups.   The "case of Krakatau" and "Protection of Nature around the Pacific" are examples of the programs of general interest, while among those of more specific interest in the field of Botany and Agriculture, may be named the following: Rice problems, breeding and selection of certain crop plants, including rubber, sugar cane, coffee, etc.; forestry problems; soil technology; and plant geography.
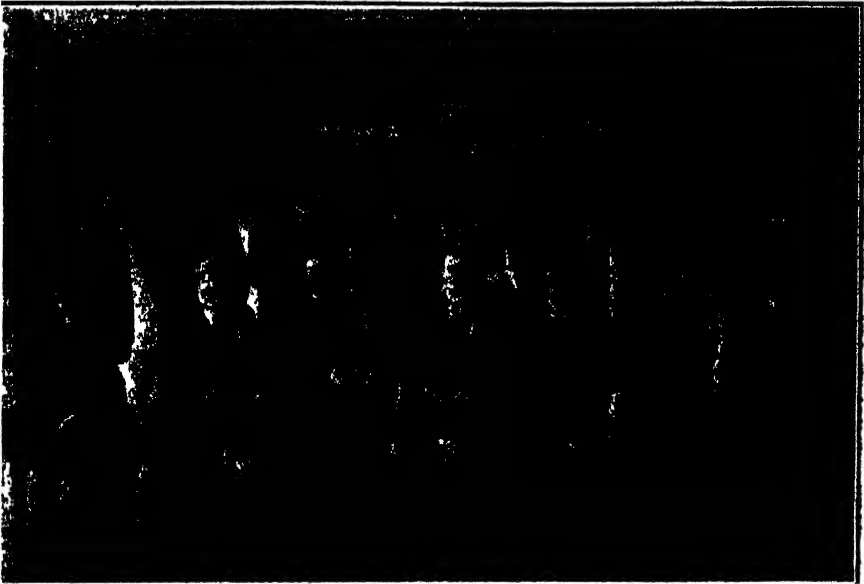
FIG. 2. Executive Committee of the Congress.

Standing, left to right: Dr. C. E. STEHN, Chief, Vulcanological Survey; Dr. J. STROOMBERG, Chief, Division of Commerce, Department of Agriculture, Industry, and Commerce; Dr. W. F. GISOLF, Petrographer, Geological Survey; Dr. S. W. VISSER, sub-director, Royal Magnetic and Meteorological Observatory; Dr. H. J. T. BYHMER, Military Surgeon and Anthropologist; Dr. K. W. DAMMERMANN, Chief, Zoological Museum and Laboratory, Buitenzorg; Dr. J. T. WHITE, Chief, Pedological Institute and General Agricultural Experiment Station.

Sitting, left to right: Dr. H. M. HIRSCHFIELD, Officer, Java Bank; Dr. W. M. DOCTERS VAN LEEUWEN, Director Buitenzorg Botanical Gardens; Dr. J. CLAY, Professor of Physics, Technical Faculty, Batavia; Dr. O. DE VRIES, Director, Rubber Experiment Station, and Professor of Chemistry on the Medical Faculty, Batavia (President of the Fourth Pacific Science Congress); Dr. H. J. LAM, Herbalist, Botanic Gardens, Buitenzorg; A. C. DE JONG, Director, Geological Survey; and Dr. J. J. B. DEUSS, Director Tea Experiment Station, Buitenzorg.

Rice is of so vast importance to the Eastern countries, that its problems were allowed considerable space on the program. In Java alone, the rice production is about 7¼ million tons per annum, grown on 8,000,000 acres. While even in Java the rice growing is entirely in the hands of the natives, the Dutch experiment stations attempt to give the industry every possible aid in improving varieties and cultural practices. The symposium on rice problems was opened by an extensive report on the economic situation by Mr. M. B. SMITS of the Division of Agricultural Economics of the Netherland East Indies, but the other papers and discussions were concerned mostly with such questions as fertilizers, the relation of temperature and

irrigation water to growth and yield, and the production of superior varieties. Fig. 3 shows rice fields near Bandoeng.

At the present time, all the rubber in the East Indies is derived from *Hevea braziliensis,* or rather varieties developed from it. Extensive investigations are being carried on in the breeding and selection of superior varieties; physiology of latex secretion; methods of tapping; preparation of the sap and its chemical composition; methods of propagation; and the combatting of pests. As a part of the selection work, records of individual tree yields are kept on all estates, and as soon as any tree shows unusual performance, it is officially recorded as a "mother tree," that it may serve later as material for further improvement.

Papers of the above nature were presented also for such crops as coffee, tea, cocòanuts, oil palms, and sugar cane.

The work on sugar cane seemed of especial value. By cultural improvements and the development of resistant varieties, the "sereh" disease has been practically conquered. In the breeding experiments aimed at the production of disease resistant and high yielding varieties, it is interesting to note that there appears to be a correlation between the number of chromosomes and the size, vigor, and sugar content of the cane.

In Plant and Animal Geography, the most popular discussions revolved around the history and development of the flora and fauna of the Krakatau Islands since the great eruption in 1883. The popularity of such discussions was greatly augmented by the delightful excursion which had been made to Krakatau, May 12 and 13. Fig. 4 shows the east side of the island, and its coastal vegetation.

The "Wallace-Weber line" drew some fire, but in the end it seemed that no distinct damage was done it.

A very interesting paper was read by Dr. von FABER, Director of the Treub Laboratory on the subject: "The Physiology of the Mangroves." A careful study had been made on these plants, both *in situ* and in experimental culture in the laboratory. The result was a detailed record of the fluctuations of the environment and the internal adjustments of the plants to those fluctuations. When a plant is removed from a nutrient solution, with addition of 2–3 per cent. NaCl to one with 8 per cent. NaCl, there is an impediment to the "suction" of the roots, so that a small deficit arises between absorption and transpiration. The water tissues begin to get thin, while assimilation leaves remain turgescent. When the latter conditions remain unchanged, transpiration is greatly reduced by the closing of stomata. The roots begin their regulating activities, the deficiency is wiped out, and transpiration becomes normal. The "saltstorers" (*e.g., Avicennia officinalis*) make this adjustment in one to two hours, and the "non-salt-storers" (*e.g., Rhizophora spp.?*) in four to five hours. When the plant is

FIG 3   Irrigated rice fields (Sawahs) near Bandoeng, Java

FIG 4   East side of Rakata or Krakatau Island in the Sunda Strait   The island
is 2300 ft high, and has reforested since 1883 to the 2000 ft line   The northwest
side of this peak is the precipitous cliff of Krakatau which is always shown in the geo
logical photographs.

returned to a 3 per cent. NaCl solution, a surplus of water accumulates and, just as at high tide, the leaves of the non-saltstorers take on a glassy appearance. But the saltstorers, through their rapid root regulation and secretion, quickly adjust themselves. Osmotic pressure is greatest at low tide at which period the leaves of *Rhizophora* have shown 148.4 atmospheres and the roots 98.6 atmospheres. The respective values for *Avicennia* were 162.2 and 96.0 atmospheres. At the end of high tide *Rhizophora* showed 77.8 and 45.4 atmospheres for leaves and roots respectively. Correspondingly, *Avicennia* showed 82.0 and 50.3 atmospheres. Experimentally *Avicennia* could be induced to develop 205 atmospheres in the leaves, with transpiration still "very liberal."

Regarding the scientific excursions of the Congress, much might be written, and it requires considerable restraint to pass them by with a mere mention of those of special interest to Botanists and Agriculturists, as follows: Krakatau Islands in the Sunda Strait; Coral Islands in Batavia Bay; the famous Botanical Gardens and laboratories (see fig. 5 for the famous Treub Laboratory) in Buitenzorg; the high altitude Botanical Garden and virgin tropical rain forest at Tjibodas on Mt. Gede, fig. 6; to the crater of the active volcano, Mt. Tankoeban Prahoe, near Bandoeng; Mangrove, swamps of the Kinderzee and Noesa Kambangan on the south coast of Java near Tjilatjap—on Noesa Kambangan is also a low altitude tropical rain forest, which is the home of the huge parasitic flower *Rafflesia patma*, fig. 7; to Madoera island off the northeast coast of Java; to the Tengger mountains at Tosori, to visit the "Tjemara" (*Casuarina junghuniana*) forests and the active volcano, Mt. Bromo; and the marvelous island of Bali—this excursion was not strictly Botanical!

The Agriculturists were given opportunity to visit several of the principal experiment stations of Java of which there are perhaps eighteen. About half of these are privately endowed. As a rule, each of the experiment stations concerns itself with but one, or only a very few products. Some of these institutions are prepared in a splendid way, both in personnel and laboratory equipment, to carry out their aims. The Sugar Experiment Station at Pasoeroean, for example, has an annual income of over $500,000.00. This is a large sum for one experiment station and a single agricultural product, but that the investigational staff is accomplishing excellent results is indicated by the experimental work on sugar cane previously referred to, and very clearly by the fact that the sugar yield in Java has gone steadily upward to the present average of 14,000 lbs. per acre. This figure stands in striking contrast to the old average in Java of 1,700 lbs. and the present average in Louisiana of only 1,300 lbs. per acre.

Another very important phase of the excursions for the Agriculturists was the opportunity to inspect numerous large estates of Cinchona, rubber, tea, coffee, teak, and other crops.

FIG. 5.   The Treub Laboratory, Buitenzorg.
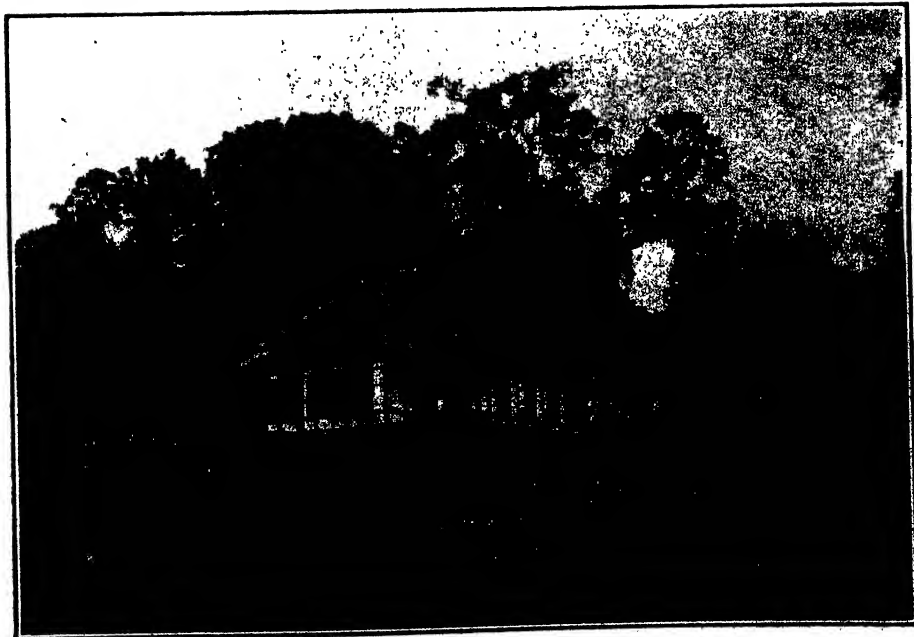
FIG. 6.   Visitors' Laboratory, Botanical Gardens, Tjibodas, at altitude of nearly
5000 ft. on Mt. Gede, Java.
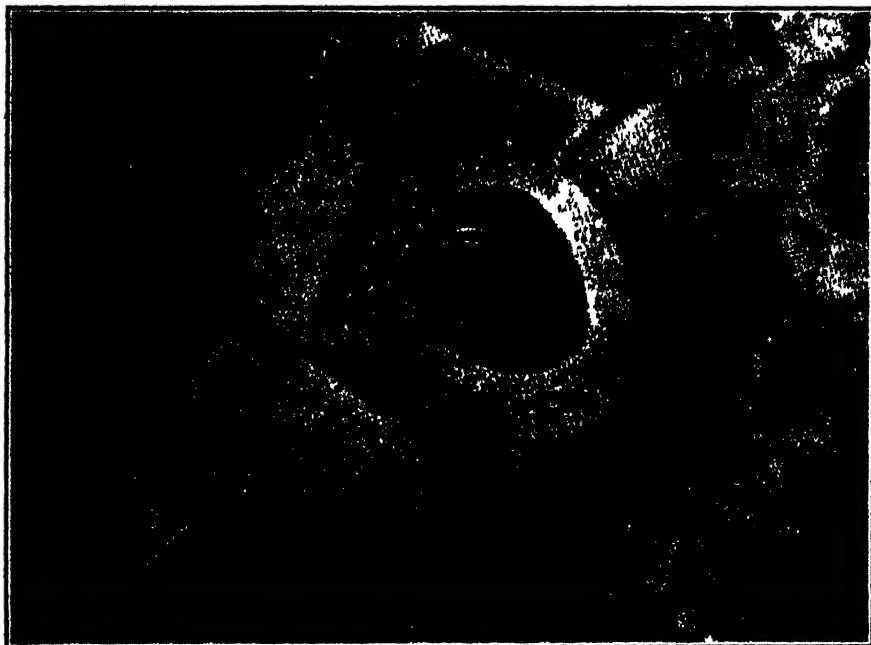
FIG. 7. Flower of *Rafflesia palma.*

The extraordinary variety of interests present in and about Java proved to be of great profit and joy to the Congress delegates. Java's interesting and modern tropical agriculture, its fauna and flora, its numerous active volcanoes and other geological situations, and its present races of men and the splendid evidences of prehistoric man, produced in the delegates a condition of general enthusiasm in which each scientific group was inclined to feel that it, somehow, held a distinct advantage over the others.

In closing this account it is a pleasure to the writer to mention again the splendid hospitality offered at all times to the delegates by the officials of the Netherlands East Indies government, and the Netherlands Science Council. The scientific value of such a Congress is admitted, but the words of His Excellency the Governor General were well taken when he said: ''I attach still more value to the results which this Congress will bear in promoting closer understanding and good will between nations and individuals.''

DEPARTMENT OF HORTICULTURE,
    OREGON STATE COLLEGE.
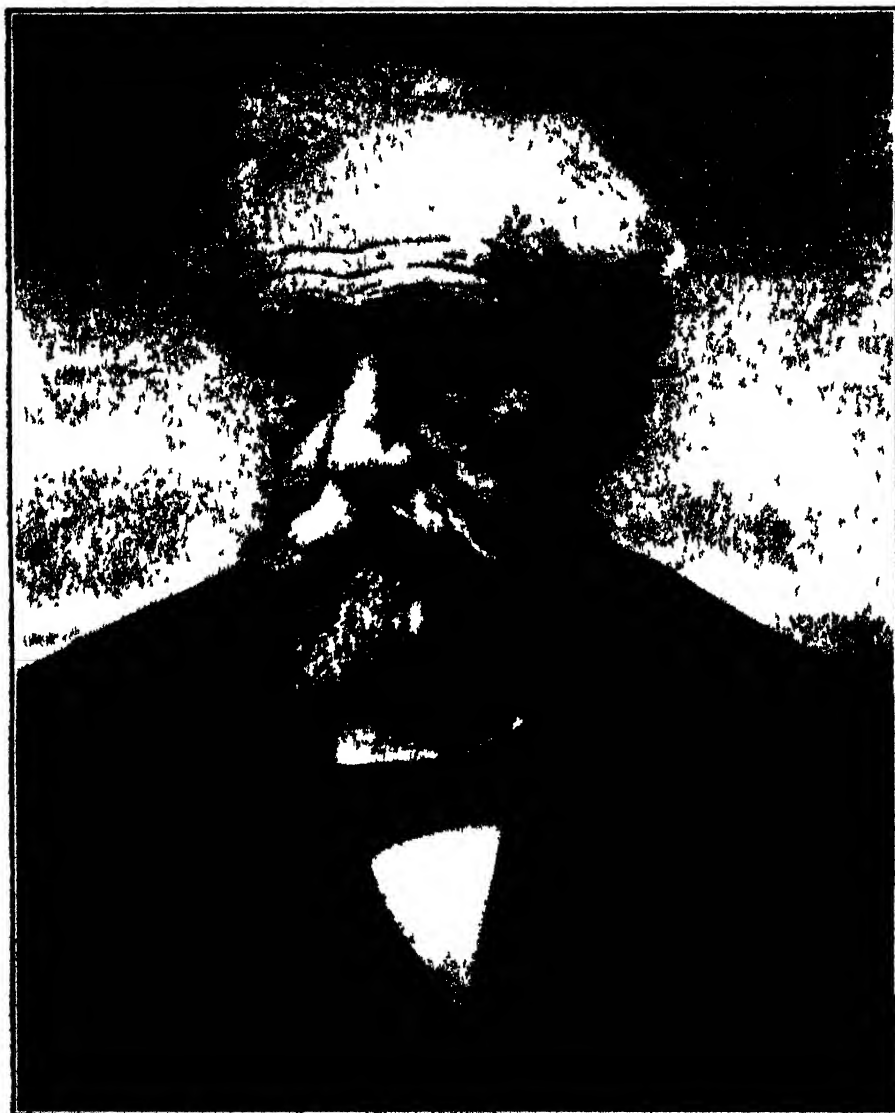
HUGO DE VRIES
1848–

BRIEF PAPERS

HUGO DE VRIES

(WITH ONE PLATE AND FOUR FIGURES)

One of the most distinguished of all botanists is HUGO DE VRIES. He studied in the University of Leiden in 1866 and afterwards in various German universities, first at Heidelberg in 1870 and then at Würzburg in 1871. At Würzburg, which was an international research center, he studied under the great plant physiologist, JULIUS VON SACHS, from whose laboratories have gone out so many scientists who have become famous. Besides DE VRIES, among these may be mentioned such other great men as PFEFFER, BREFELD, NOLL, STAHL, ELFVING, KLEBS, GOEBEL, G. KRAUS, F. DARWIN, and many others. SACHS exerted a great influence on the scientific thought and work of DE VRIES and many other investigators.

Professor DE VRIES occupied the chair of botany at the University of Amsterdam for many years. He was born at Haarlem, February 16th, 1848. His first paper, entitled "De invloed der temperature ob de levensverschijnselen der planten," appeared in 1870. Since the appearance of this paper other very important contributions have followed one another through the years in rapid succession. Many of the papers of DE VRIES have been collected by some of his students and published in seven large volumes under the title of "Opera e periodicis collata" (1918 to 1927). These volumes contain 189 of his contributions, of which the above mentioned paper is the first. The seven volumes included almost 4300 pages, which is only a part of the large amount of scientific work he has done. Professor DE VRIES has published his studies in four different languages. Some of his papers collected in "Opera e periodicis collata" are in the Dutch language, part of them in German, some in French, and the rest in English. Some of his contributions, however, are very extensive and therefore have been published in book form. Among these may be mentioned "Intracellulare Pangenesis," of which an English translation has appeared; and his great Mutationstheorie, 1901–1903, which appeared in German, in two large volumes. This work was soon translated into English. "Species and Varieties, Their Origin by Mutation" appeared in 1905. These and other important contributions appeared in book form and his "Species and Varieties" in more than one edition in English. A great service was rendered by DE VRIES since, in 1900, along with CORRENS and TSCHERMAK, he re-discovered MENDEL's long forgotten work. The plate and fig. 1 are excellent likenesses of him from recent photographs.
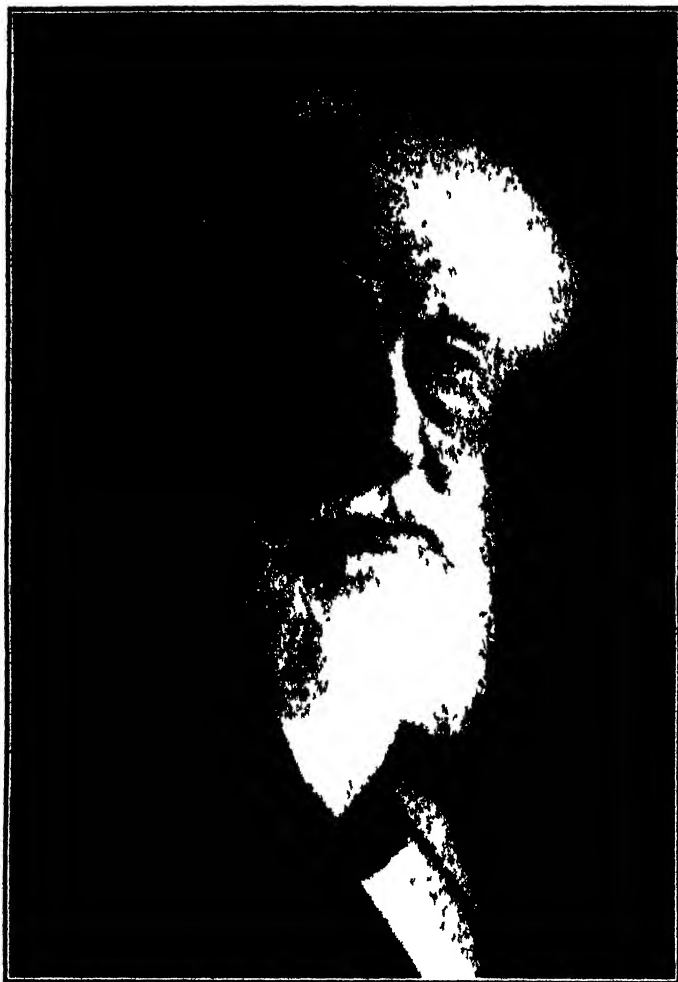
Fig. 1. Professor Hugo de Vries (recent photograph).

The first studies of Professor DE VRIES were concerned with plant physiology, in which subject he published many valuable papers. These contributions are of such unusual importance and are so well and favorably known that a mere mention of the titles of a few of them is sufficient. In addition to those contributions mentioned or referred to elsewhere by the writer in this paper, the results of his studies on plasmolysis, turgor, growth, osmosis, and other topics are of such far reaching importance as to place him among the most distinguished scientists of his day.

The importance of the distinguished work of DE VRIES is attested by the recognition accorded him by universities and scientific societies all over

the world. Some of the universities that have bestowed upon him the honorary degrees of Doctor of Science and of Laws are: Columbia University, and the Universities of Chicago, Pennsylvania, Cambridge (England), and Aberdeen (Scotland)

Many Academies of Science have honored him by membership as follows: The Dutch Academy of Science since 1878; Associate Member of the Royal Academy of Belgium, 1905; a Corresponding Member of the Institute de France, 1913; Ehrenmitglied der deutschen botanischen Gesellschaft, 1891; Ehrenmitglied der k. Akademie der Wissenschaften in Wien,

FIG. 2. Professor DE VRIES in his experimental garden.

1919; Corresponding member of the Prussian Academy of Sciences of Berlin, 1913; Foreign Member of the Royal Society, London, 1905; Member of the Academia dei Lincei Rome, 1902; and Corresponding Member of the Russian Academy of Sciences, 1925.

Various societies in the United States have honored him by membership for his scientific achievements. He is a Member of the American Philo-

sophical Society, Philadelphia, 1907; a Corresponding Member of the Academy of Natural Sciences, Philadelphia, 1903; Foreign Associate of the National Academy of Sciences of the United States of America, Washington, 1904; Corresponding Member of the Botanical Society of America, 1922; Honorary Member of the American Breeders Association, Washington, 1910; Honorary Associate of the Station for Experimental Evolution at Cold Spring Harbor of the Carnegie Institution of Washington, 1904; Professor DE VRIES was present at the opening of this station and delivered on that occasion an address on the "Aims of Experimental Biology."

Various medals have also been awarded to Professor DE VRIES as follows: The Darwin Medal by the Royal Society, London, 1906; The Veitsch Medal, by the Royal Horticultural Society, London, 1910; and the Linnaean Gold Medal by the Linnaean Society, London, 1929.

FIG. 3.   Garden with *Oenothera* in bloom.

On the 16th of February, 1918, the Berichte der deutschen botanischen Gesellschaft sent him its greetings.   He was then 70 years old and I quote two sentences only from that message, which are self-explanatory.

"Mit zwei Arbeitsgebieten der Botanik wird ihr Name für immer verknüpft bleiben.   Waren es zunächst Fragen der physikalischen und chemischen Physiologie, wie des Turgordruckes, der Plasmolyse, der Mechanik des Zellwachstums, denen Ihre Hauptarbeit gegolten hat, so ist es später vor allem das Gebiet der Vererbungs- und Artbildungslehre auf dem

Sie bahnbrechend tätig waren und noch tätig sind.'' On his eightieth
birthday in 1928 scientists from all over the world sent him messages and
greetings.

Professor DE VRIES has visited the United States on three occasions.
On two of these visits he delivered lectures at the University of California
and the University of Chicago. His third visit to this country was in 1912
when he was invited to speak at the formal opening of the Rice Institute.
While there he gave four lectures.
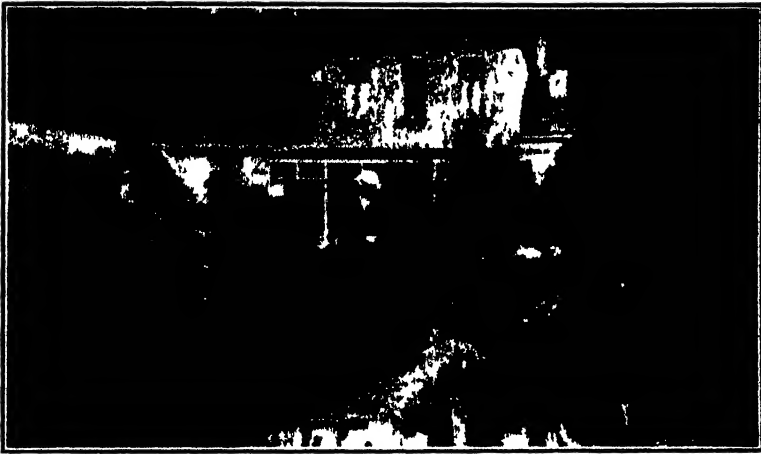
FIG. 4. Professor DE VRIES examining plants in his garden at Lunteren.

Figure 2 shows Professor DE VRIES in his experimental garden among
the plants he is studying. In the photograph one can see a part of two
of the flower beds in which the plants are grown, and at the right are to
be seen four tall primrose plants whose tops are covered with paper sacks
which are used in the pollination experiments.

Professor DE VRIES has been emeritus professor at the University of
Amsterdam since 1918. He now resides at Lunteren, Holland. Here he
has his garden, which, as shown in fig. 3, is full of *Oenothera* plants in
bloom. The garden belongs to Professor DE VRIES and is part of the garden
around his home. On the right side of fig. 3 is shown a portion of the
garden enclosed with glass, and fig. 4 shows another view of the garden at
Lunteren. It is in this part that most of the fertilizations and crossings
of the experimental plants are made. Much of the material used by the
recent students of Professor DE VRIES has been obtained from this garden
for their studies.[1]

[1] The author is indebted to Professor DE VRIES for information concerning various
points in this paper.

Two of the latest papers of Professor DE VRIES indicate his present scientific interests. These papers are: "Mutant races derived from *Oenothera lamarckiana semigigas*" and "Die latente Mutabilität von *Oenothera biennis* L."

Although past 81 years of age, Professor DE VRIES is still actively interterested and is diligently at work in his chosen field and continually adding to the long list of valuable contributions already made. His long and active life is an inspiration to two generations of younger investigators. Two great services he has rendered to science are first, that he has shown that mutations occur, and secondly, that in his investigations he has arrived at his results and conclusions by most careful experimentation.—F. M. ANDREWS, *Indiana University, Bloomington, Indiana.*

NOTE ON THE RELATION OF RATE OF RESPIRATION TO CHEMICAL COMPOSITION IN FRESH VEGETABLES

In a recent publication from this station,[1] the rates of respiration of some common vegetables during the first thirty hours after harvesting, were compared. Samples of the same material were prepared for analysis by careful drying, and with the exception of the phosphorus fractions[2] standard analytical procedures were used. It was thought that, although these vegetables represented different organs, and might naturally be expected to respire at different rates, there might be certain relationships between the rate of respiration and the chemical composition which would transcend the lines of demarcation laid down by function. In an attempt to detect such relationships, suggested, or even vaguely possible, the analytical data were plotted in a variety of ways together with the respiration figures. But we were unable to discover any relationships which ran consistently through the series.

Soluble nitrogen seemed to be directly related to respiration in the case of asparagus, green beans, okra, green onions, and carrots; with the other vegetables, the curve for this relationship was very irregular. Ether extract and lipoid phosphorus showed a nearly constant value for all the vegetables, without relation to respiration. The reducing-sugar values, which might have been expected to run parallel with the respiration curve, were extremely variable, as were also the starch values which might have been expected to bear an inverse relationship to respiration.

We realize that figures obtained from only ten samples do not constitute conclusive evidence one way or the other, and that there may be relationships here which our work fails to bring out.

The data are presented in the accompanying table. The weight of carbon dioxide evolved in 24 hours (from the second to the twenty-sixth after harvesting) per 100 grams dry weight, is taken in each case as the measure of the rate of respiration. The other figures are also on a dry weight basis.—MARJORIE P. BENOY and JAMES E. WEBSTER, *Oklahoma Agricultural Experiment Station.*

[1] BENOY, MARJORIE P. The respiration factor in the deterioration of fresh vegetables at room temperature. Jour. Agr. Res. 39: 75–80. 1929.

[2] WEBSTER, JAMES E. Phosphorus distribution in grains. Jour. Agr. Res. 37: 123–125. 1928.

TABLE I

ANALYSES OF VEGETABLES, AND CO_2 PRODUCTION PER 100 GRAMS (DRY WEIGHT) DURING 24 HOURS

PERCENTAGES ON DRY WEIGHT BASIS

DETERMINATION	ASPARA-GUS	LETTUCE	GREEN BEANS	OKRA	GREEN ONION	CARROT	TOMATO	BEET	GREEN SWEET PEPPER	PIMENTO
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Dry matter in original material	8.34	6.16	7.67	13.66	9.34	13.91	6.13	9.51	8.01	9.38
Ash (dry basis)	7.84	17.58	7.66	6.54	9.73	13.17	10.38	19.75	7.05	9.51
Total phosphorus	0.70	0.49	0.53	0.38	0.34	0.45	0.50	0.28	0.43	0.55
Inorganic phosphorus	0.20	0.15	0.12	0.10	0.19	0.16	0.21	0.12	0.19	0.23
Lipoid phosphorus	0.05	0.05	0.04	0.04	0.07	0.05	0.04	0.05	0.05	0.05
Organic phosphorus	0.50	0.34	0.41	0.27	0.15	0.29	0.29	0.16	0.25	0.32
Total nitrogen	4.75	2.94	3.32	2.65	2.57	2.06	3.89	2.97	2.87	3.05
Insoluble nitrogen	0.94	----	2.50	1.76	1.83	1.77	1.07	1.61	1.37	1.05
Soluble nitrogen	3.80	----	0.82	0.89	0.74	0.28	2.79	1.36	1.50	1.99
Total sugars	35.88	8.92	24.65	12.30	22.81	12.01	39.58	8.63	32.07	36.03
Reducing sugars	33.21	5.68	24.65	11.57	20.99	8.20	39.58	5.58	31.95	31.66
Starch	1.54	1.61	4.97	18.75	0.77	6.03	1.12	0.86	5.02	1.15
Total carbohydrates	37.42	10.53	29.62	31.05	23.59	18.04	40.70	9.49	37.09	37.18
Ether extract	4.11	3.55	3.00	2.04	4.12	3.10	4.29	3.07	2.97	5.47
Weight CO_2 evolved in 24 hours per 100 grams dry weight	20.066	9.424	9.273	7.669	6.627	4.659	3.92	2.958	2.88	1.893

THE PHYSIOLOGICAL LABORATORY OF THE BOTANICAL
INSTITUTE IN THE UNIVERSITY OF ZAGREB

A new Institute for Plant Physiology (fig. 1) has been established at the University of Zagreb, Jugoslavia, under the direction of Dr. VALE VOUK. The new laboratory is associated with the work in forestry, agriculture, pharmacology, and other scientific departments of the University, and also with the International Corn Borer Investigations Laboratory at Chicago.

At the dedication of the new laboratory on December 18, 1928, Dr. VOUK in his address reviewed the developments of plant physiology at the University of Zagreb and also the general history of the science in other countries.

The Institute comprises experimental gardens, greenhouse, laboratory, and library space. It was erected at a cost of approximately $20,000.— R B. HARVEY, *University of Minnesota.*

FIG. 1.   The new laboratory for plant physiology at the University of Zagreb, Jugoslavia.

NOTES

The Annual Meeting.—The American Society of Plant Physiologists met at Des Moines, Iowa, on December 30 and 31, 1929, and January 1, 1930, for its sixth annual meeting. Dr. S. V. EATON, of the University of Chicago, presided over the meetings, assisted by Dr. A. E. MURNEEK, of the University of Missouri, vice president of the Society. The programs were well arranged and well organized, and nearly all of the papers were presented by the authors in person. Only a few papers were read by title only. The joint meeting with the Horticulturists on Tuesday morning at Des Moines, and with the Ecologists at Ames, were very pleasant occasions.

The secretary-treasurer, Dr. H. R. KRAYBILL, of Purdue University, presented his report for the fiscal year ending June 30, 1929, and the statement of the current condition of the treasury. The membership report showed a much larger increase than in either of the last two preceding years, but the increase in institutional subscriptions was not so great as during last year. However, the membership is approaching four hundred, and there are about 250 institutional subscriptions at the close of 1929. The treasury showed a very comfortable margin for 1929, and income for 1930 sufficient to maintain or even improve the publication service. It may be possible to bring out a new edition of the International Address List, which was first issued in 1925.

The banquet on Monday night, December 30, at the Savery Hotel, was attended by about 85 members and friends of the Society. The annual dinner has been featured for several years by the announcement of the awards of honors, which makes it one of the most interesting sessions of the meeting. The meeting of the Society for December, 1930, will be held at Cleveland, Ohio. By looking ahead and planning for larger things, we can make each annual meeting set a new and higher standard of achievement.

Life Membership Awards.—For the fourth time the Society has awarded life memberships to some of its most distinguished members as a memorial to Dr. CHARLES REID BARNES. The committee of award, with Dr. WALTER THOMAS, of Pennsylvania State College, as chairman, made two awards. The first one was to Professor GEORGE JAMES PEIRCE, for many years Plant Physiologist at Stanford University. Professor PEIRCE was born at Manila, Philippine Islands, in 1868. His scientific training was taken at Harvard, and at the Universities of Bonn, Leipzig, and Munich. His doctor's degree is from Leipzig in 1894. After two years of service at Indiana University, he went to Stanford in 1897, where he has given more than 30 years of his life to the advancement of plant physiology. His Text

Book of Plant Physiology was published in 1903, and a more recent book, The Physiology of Plants, in 1925. He has been interested in respiration, irritability, parasitism, and brine-inhabiting organisms. He has held various positions as special investigator of the effects of gas, smoke, and dust on vegetation, and was a member of the Advisory Committee to the Fuel Administration of California during the late war.

There was a bond of mutual friendship between Dr. BARNES and Dr. PEIRCE in the early days of plant physiology in the United States, and it seems very fitting that the award should be made to one of these early friends of BARNES.

The second award was made to Professor CHARLES A. SHULL, of the University of Chicago, who was at one time student assistant in Dr. BARNES's office, and who received his first instruction in plant physiology under Dr. BARNES in 1903–1906.

This action of the committee gives the Society five honorary life members, who constitute a living memorial to him who was the main inspiration of plant physiologists in the United States during the first decade of this century.

Stephen Hales Award.—The first award of the STEPHEN HALES Prize was also announced at the banquet. The Committee, consisting of Dr. J. B. OVERTON, chairman, Dr. C. R. BALL, and Dr. A. L. BAKKE, after consideration of many of the questions involved in the selection of candidates for this honor, finally made the award of the certificate and prize of $100 to Professor DENNIS ROBERT HOAGLAND, of California, for his excellent contributions and leadership in the field of plant nutrition. Professor HOAGLAND has been at the University of California for 17 years, and under his leadership the plant nutrition laboratory at California has made many important contributions, particularly with reference to the mineral nutrition of plants.

The certificate of award bears a handsome likeness of STEPHEN HALES, the first great experimentalist in the field of plant physiology. The plan of administration of the award contemplates an address by the recipient of the award before the Society at the following annual meeting.

The executive committee, on information from the secretary-treasurer as to the funds available for a STEPHEN HALES award on November 1, 1929, voted a second award to be made at the annual meeting at Cleveland in 1930.

The Charles Reid Barnes Life Membership Fund.—The Society, at the recommendation of the executive committee, has taken favorable action on a plan to create a permanent CHARLES REID BARNES Life Membership Fund

to replace ultimately the present temporary financing. The plan adopted is to establish such a permanent fund to which individual gifts may be made. In addition, any of the life membership funds vacated by the death of life members are to be put into the permanent fund until the total of such private gifts and vacated funds reaches the sum of $2000. This fund will then be known as the CHARLES REID BARNES Life Membership Fund, the income of which will be employed in the same manner as is the income from the present temporary fund.

Individuals, some of whom have expressed a desire to share in the creation of this permanent fund, should now avail themselves of the opportunity. Checks may be sent at any time to Dr. H. R. KRAYBILL, Purdue University. Or, the purchase of a life membership by those who can afford to make the investment, will now give the investor his official journal for life, and finally assist in the creation of this permanent fund in honor of Dr. BARNES. There is no better way to aid than by the purchase of life memberships. They can be paid in installments, but do not become operative until paid in full.

The Stephen Hales Prize Fund.—This fund is slightly less than $1000. It ought to be larger, as it requires not less than $1000 to produce the amount of the prize every two years. The funds are now invested in bonds yielding above the average interest. When reinvested it is doubtful if conditions will warrant renewal at the present rate. There are now about four times as many members in the Society as the number who contributed the $991.75 which constitutes the fund. Many small gifts are more desirable than a few large ones. It is not improbable that members will be offered the privilege of sharing in the enlargement of this fund. A 50 per cent. increase would insure the award as at present planned, and would permit increases in the amount of the biennial award, or would make more frequent awards possible.

General Endowment Fund.—The Society voted at the Des Moines meeting that a general endowment fund would be a desirable thing. At present it would hardly be possible to undertake publication of papers which demand colored plates for proper presentation of results. It usually costs several hundred dollars for a single colored plate. Moreover, as PLANT PHYSIOLOGY serves a larger and larger membership, there is the possibility that the size of the volume would finally be limited by the income, so that worthy contributions could not be given prompt publication. Endowment funds protect an organization from such difficulties, and make possible types of service beyond that of the ordinary journal. The finance committee is authorized to plan for the beginning of an endowment fund. If any mem-

ber believes that PLANT PHYSIOLOGY should, when necessary, use colored plates, or undertake other extraordinary services in publication, a gift to the general endowment fund, even though small, will help toward the achievement of such services. It is hoped that every member will take an interest in these funds, and will try to share, as fortune permits, in the privilege of founding these financial bulwarks of the Society. Any gift sent to the secretary-treasurer, or to the members of the finance committee, should be plainly designated to some particular fund. Every gift will further the purposes of the Society and help it to give a better service to its members.

Travel to the International Botanical Congress.—Some of the members of our Society will be going to Cambridge next summer. This notice is to call attention to the service of the Student Third Cabin Association. It offers a means of going and coming that may interest many of us. It is a special Tourist Third Cabin on the Holland-America Line ships which was created by two Yale students six years ago. It differs from other Tourist Third Cabins in being maintained entirely for college people and those with whom they naturally associate. In addition to most congenial fellow passengers on the ocean, it offers cabins, decks and public rooms that were formerly second class on the steamers Rotterdam, New Amsterdam, Volendam, and Veendam, and remarkable Tourist Third Cabin accommodations on the new Holland-America Line flagship Statendam. The service on these vessels is all that could be desired, the food is first class, well prepared and simply served, and everything is kept scrupulously clean.

Sailings are each Saturday from New York. The ships of this line call at Plymouth in England going to Europe, and leave from Southampton on the return voyage. In France, the port both ways is Boulogne-sur-Mer, two and one half hours from Paris. The other European port is Rotterdam, Holland, the gateway to Central Europe.

The parties crossing are provided with a host and hostess, a lecturer and college orchestra, and a loan library on all of the larger STCA sailings. A guide book is obtainable, called the Hand-Me-Down, that is one of the best for European travel, and there is a complete travel department that can help individuals or groups to plan anything desired in the way of travel experience while abroad. Information on renting Drive-yourself cars in Europe will be furnished. There is no charge for such service except for the Hand-Me-Down.

A number of single, double, and four berth cabins have been reserved on each sailing in June, July and early August with corresponding returns for people going to the Congress. Those who are planning to go should investigate this plan, as it is less expensive than many, and leaves more

money to spend abroad. Full information will be furnished to any one, on sending inquiry to the Student Third Cabin Association, Holland-America Line, 24 State St., New York City.

Dr. Felix Kotowski.—We regret to announce that one of our foreign members has been claimed by death. The following statement concerning the life of Dr. FELIX KOTOWSKI has been prepared from data furnished by Dr. J. GOLINSKA, a colleague, and by W. W. BRIERLEY, Secretary of the General Education Board, New York.

Dr. KOTOWSKI was born on May 18, 1895, in Grabova, near Radom in central Poland. He was educated in Poland, and was a student in the Division of Biological Science and Agriculture in the University of Krakow from 1913 to 1918. He received the degree of Doctor of Philosophy from the Jagellon University in Krakow in 1919. During his student days he was Assistant in the Institute of Soil Management and Plant Cultivation in the University, 1917–1918. After graduation, and until 1922, he was assistant in the Horticultural Department of the State Scientific Institute at Pulawy. In January, 1922, he was appointed Professor of Olericulture at the College of Agriculture, Warsaw, and Director of the Institute of Olericulture and Vegetable Breeding at Skierniewice, near Warsaw. This position he held until July, 1926, at which time he was granted a fellowship for study in the United States by the International Education Board. During the period of the fellowship, to September, 1927, he worked at the University of California, Davis, California, in the Division of Truck Crops, under the supervision of Professors H. A. JONES and J. T. ROSA. The results of the year's work have been published in the Proceedings of the American Society for Horticultural Science, 1926, and in PLANT PHYSIOLOGY, 1927. While in the United States he also visited the experiment stations at Ithaca and Geneva, New York, to familiarize himself with their investigations of vegetable crops. He returned to Poland by way of the Pacific, in order to visit experiment stations in Hawaii, Japan, Ceylon, and India.

Dr. KOTOWSKI was very industrious, and labored to secure a universal scientific education. During the ten years of his scientific career, he published about 40 papers on the morphology, physiology, and breeding of vegetable plants. He was the author of a text-book of horticulture, and several popular publications on different horticultural questions. He was also one of the leaders in organizing the olericultural experimental work in Poland.

He died on July 9, 1929, of blood-poisoning caused by a scratch of the lower lip. The untimely death of Professor KOTOWSKI at the age of 34

years is a great loss to Polish horticultural science. His students have lost a brilliant guide, and a real friend.

Back Numbers Wanted.—Volume 2 of PLANT PHYSIOLOGY is practically exhausted. The limiting factor is no. 4, the October issue, 1927. Members and subscribers are urged to examine their files, and if duplicates of this number are found, the Secretary-treasurer would like to purchase copies of this number to complete broken volumes. Any one willing to dispose of this particular number should write to Dr. H. R. KRAYBILL, Purdue University, Lafayette, Indiana. Cooperation of the members and subscribers in this matter will be greatly appreciated.

Pathology of Protoplasm.—The third volume of Protoplasma-Monographien is entitled "Pathologie der Pflanzenzelle." Part I bears the title "Pathologie des Protoplasmas." The author is Dr. ERNST KÜSTER, of Giessen. The discussion is presented in two chapters, one on Formwechsel, the other on Strukturwechsel. The character of the material presented is such that it should hardly be called pathology. It really presents some interesting phases of the physiology of protoplasm, and plant physiologists will find the monograph worth reading for a better understanding of protoplasmic responses to all kinds of environmental stimulations. Over 500 references to the literature are included at the close. The publishers are the Gebrüder Borntraeger, W 35 Schöneberger Ufer 12 a, Berlin, Germany. The price of the volume bound in cloth is 15 M.

Problem of Krakatao.—The problem of the revegetation of Krakatao after the destructive eruption of August 27, 1883, has been given a refreshingly critical treatment by Dr. C. A. BACKER, formerly government botanist for the flora of Java. BACKER takes sharp issue with the dictum of TREUB that the explosion completely destroyed the original vegetation of the island. He shows how inadequate all of the investigations have been, and how the opportunity to study this interesting problem of revegetation was forever let slip.

The book has 12 chapters and 299 pages. It is published by the author, but can be purchased from Martinus Nijhoff, The Hague, Holland. The price, bound, is 9 guldens, plus postage.

Soil Acidity.—An excellent monograph on soil acidity by Dr. H. KAPPEN has appeared from the press of Julius Springer, Berlin. "Die Bodenacidität" contains fifteen chapters, as follows: Nature of acidity of mineral soils; soil reaction; determination of soil reaction; behavior of acid

soils toward acids and bases—their neutralization or buffer capacity; behavior of acid soils toward solutions of salts (hydrolytic, exchange, and active acidity, etc.); absorption power of acid soils; meaning of acidification for physical soil characters; influence of reaction on the microorganisms of the soil; the plant physiological meaning of soil reaction; occurrence and distribution of soil acidity; influence of manures on soil acidity; the prevention of acidity injuries by liming; and the use of artificial fertilizers on acid soils.

There are 363 pages, 35 text figures, and one colored plate. This work will be quite valuable to the student of soil acidity problems. The price unbound, is 36 M., bound, 38.80 M.

International Critical Tables.—Volume VI of this great work was issued late in 1929 by the McGraw-Hill Book Co. The general nature of the contents of this volume was indicated in PLANT PHYSIOLOGY **4**: 295. 1929. The following data are included: X-ray, electronics and gas conduction, dielectric properties, electrical conductivity and resistivity, pyro- and piezo-electricity, thermoelectricity, transference numbers of electrolytes in aqueous solutions, electrolytic electromotive force, electrical and optical properties of SiO_2, magnetism, atmospheric electricity, terrestrial magnetism, and acoustics. The work is immensely valuable to all fields of science. The final volume is due sometime during 1930.

Plant Ecology.—The new text-book on Ecology by Dr. J. E. WEAVER, of Nebraska, and Dr. F. E. CLEMENTS, of the Carnegie Institution, is a notable attempt to provide a good systematic survey of this field. Plant physiologists should find this volume particularly welcome as a text in Ecology, as it is the best attempt so far to give ecology a balanced physiological setting. We should all be interested in the field aspects, as well as the laboratory aspects of our field, for plant behavior does not cease to be physiological just because it occurs in the open rather than under glass or in a laboratory; and all plant physiologists will be better physiologists by knowing something of the vegetational phenomena of the earth. The parts particularly valuable to the physiologist are those chapters and sections that deal with the soil, underground plant parts, adaptation, germination of seeds, dormancy, summation of temperatures, tolerance, effects of duration of light, etc.

This book is so far superior to anything else available for the study of ecology as to leave it in a field by itself. The authors and publishers have prepared a book that will no doubt have a deep influence upon the study of plant life. The work is sold at $5.00, and familiarity with its contents will prove a good investment of time and money. Orders for it can be sent to the McGraw-Hill Book Co., New York.

Plant Competition.—Publication no. 398 of the Carnegie Institution, Washington, D. C., entitled "Plant Competition, an Analysis of Community Functions," is by Dr. F. E. CLEMENTS, Dr. J. E. WEAVER, and Dr. HERBERT C. HANSON. It is an experimental analysis of the competition of plants in the plant community, and the plant reactions which come from such competition. Some new methods have been devised for a comprehensive study of the phenomena, and studies are made of natural communities, crops, and greenhouse controls. Many of the results are of great value to the physiologist.

The first section develops the history of the competition concept, following which are sections dealing with transplant cultures in subclimax prairies and true prairies, with supplementary studies of prairie competition. Competition in prairie-woodland tension zones and in cultivated fields are considered, and the relative importance of the various factors in competition. The last two sections deal with functional studies in control cultures, and the nature and rôle of competition.

There are 32 plates, a few colored to distinguish root systems in competition. Including a lengthy bibliography, the book contains 340 pages. It is an important contribution to the general problem of competition among organisms. It can be purchased from the Carnegie Institution for $3.25, or $4.25 in cloth binding.

PLANT PHYSIOLOGY

APRIL, 1930

ASH CONSTITUENTS OF PASTURE GRASSES, THEIR STANDARD ELECTRODE POTENTIALS AND ECOLOGICAL SIGNIFICANCE

H. P. COOPER

Since there is a wide variation in the ash constituents of a species of plant grown under various conditions, it was decided to determine the ash constituents of several pasture grasses grown on soils differing widely in productivity. An examination of some available data on ash analyses of pasture plants suggested that the only way to secure definite information on this important topic was to attempt to determine, by correlating the existing data, the order of absorption and the condition necessary for optimum assimilation of the various nutrient ions.

The lack of a close correlation between the H-ion concentration of the soil and plant growth suggested a consideration of the potential relations which might result from the presence in the soil complexes of electropositive elements above and below hydrogen in the electromotive series. Such a consideration soon made it evident that the electromotive series could be used very effectively in correlating much of the data in the field of nutrition. It was observed that there is a direct relation between the order of removal of ions from soil colloids by electrodialysis, and the order of atomic ions in the electromotive series (19, 20). It was also observed that many organisms selectively absorb atomic nutrient ions in the same order as they appear in the electromotive series. Observations on the selective absorption of the strong ions led to a consideration of the radiant energy necessary for the optimum assimilation of the various nutrient compounds. A consideration of thermo-dynamical data clearly showed that the physiological effects of radiant energy are definitely related to the decompositional voltages, or processes in which a change of valence takes place, of nutrient materials. Since so much of the data on physiological problems can be correlated with the electromotive series it is very probable that this series

could be used more generally in the interpretation of data which have been obtained in biological investigations.

Selection of material for ash analysis

Many published analyses of pasture plants represent mixed herbage, including grasses and clovers. In such investigations it would probably be more desirable to select grass samples from situations where one grass predominated. Since there is a more or less definite succession of pasture plant associations accompanying the depletion of soils, it was decided to select samples from the four major associations found in New York pastures. Kentucky blue grass usually dominates on pastures which are in a high state of fertility, and as soon as the soils become depleted of the elements which are characteristic of a high state of fertility, Kentucky blue grass may be followed by Rhode Island bent grass or red top. On further depletion of the soil, sweet vernal grass may dominate, and finally poverty grass, weeds and trees may represent the dominant type of vegetation.

The samples from these four associations were taken when the grasses were in full bloom or shortly after. The Kentucky blue grass and sweet vernal grass samples were collected between the middle of June and the first of July. The Rhode Island bent grass and poverty grass samples were obtained three to four weeks later, since these do not bloom until relatively late in the season. Soil and subsoil samples were taken for hydrogen-ion determinations at the time the grass samples were collected.

Ash analyses of pasture grasses

The ash analyses of pasture grasses which were made in the Agronomy laboratory are given in table I. The data on Kentucky blue grass, Rhode Island bent grass, sweet vernal grass and poverty grass are from common pasture plant associations found in New York. The data on Canadian blue grass, broomsedge and rice cut-grass do not represent typical pasture conditions. These latter samples were selected to demonstrate the tolerance of plants to various ecological conditions.

The nitrogen and the ash content of the plants seem to decrease as the soils become depleted. Potassium oxide is seen to constitute from about one-third to two-fifths of the ash content of the common pasture grasses. It is relatively high in Kentucky blue grass and sweet vernal grass, but it is relatively low in Rhode Island bent grass and poverty grass. The calcium oxide content is highest in Rhode Island bent grass and poverty grass. This reverse relationship between the potassium and calcium contents seems to be related to the season of the most rapid growth period of these plants. The plants with their rapid growth period early in the season such as Kentucky blue and sweet vernal grasses are relatively high in potassium

TABLE I

NUTRIENT ELEMENTS IN THE ASH OF PASTURE GRASSES REPRESENTING THE VARIOUS PLANT ASSOCIATIONS CHARACTERISTIC OF THE DIFFERENT SOIL FERTILITY LEVELS FOUND IN NEW YORK

GRASS	NUMBER OF SAMPLE	AVERAGE pH OF SOIL	PER CENT. K_2O		PER CENT. CaO		PER CENT. MgO		PER CENT. P_2O_5		PER CENT. SiO_2		N PERCENTAGE		ASH
			PLANT	ASH	PLANT	ASH	PLANT	ASH	PLANT	ASH	PLANT	ASH	PLANT	ASH	PERCENTAGE
Kentucky blue (*Poa pratensis*)	9	6.79	2.36	39.07	0.18	2.98	0.18	2.98	0.57	9.44	1.91	31.62	1.51	25.00	6.04
Rhode Island bent (*Agrostis tenuis*)	12	5.18	1.44	25.90	0.33	5.93	0.20	3.60	0.39	7.01	2.55	45.86	1.33	23.92	5.56
Sweet vernal (*Anthoxanthum odoratum*)	6	5.28	1.84	41.34	0.16	3.59	0.12	2.69	0.41	9.21	1.15	25.84	1.11	29.94	4.45
Poverty (*Danthonia spicata*)	5	5.58	1.45	39.29	0.27	7.32	0.13	3.52	0.25	6.77	1.18	31.98	0.88	23.85	3.69
Canadian blue (*Poa compressa*)	4	7.64	1.18	32.67	0.28	7.76	0.14	3.88	0.22	6.09	1.34	37.12	0.75	20.77	3.61
Broomsedge (*Andropogon virginicus*)	1	4.74	0.40	7.78	0.19	3.70	0.05	0.97	0.30	5.84	4.21	81.91	0.71	13.81	5.14
Rice cut-grass (*Leersia oryzoides*)	2	5.17	0.51	5.12	0.21	2.11	0.17	1.72	0.43	4.32	8.15	81.91	1.18	11.86	9.95

and low in calcium while the plants with their rapid growth period late in the season are relatively high in calcium and low in potassium. The per cent. of phosphorus in the ash seems to be positively correlated with the potassium and negatively correlated with the calcium content. The silica content of the hard and unpalatable grasses such as Rhode Island bent grass, broomsedge and rice cut-grass is relatively high.

The correlations between the various constituents are given in table II. They represent the percentages of the entire plant with the exception of the correlation -0.45 ± 0.08 for P_2O_5 and SiO_2 which represents the percentage of these elements in the ash of the plants.

TABLE II

CORRELATION COEFFICIENT FOR THE NITROGEN AND ASH CONTENT OF FORTY-FIVE SAMPLES OF PASTURE GRASSES

K_2O and CaO	-0.87 ± 0.023
K_2O and MgO	0.17 ± 0.11
K_2O and P_2O_5	0.76 ± 0.043
K_2O and N	0.76 ± 0.043
K_2O and ash	0.65 ± 0.06
CaO and MgO	0.45 ± 0.08
CaO and P_2O_5	-0.44 ± 0.08
CaO and N	0.001
CaO and ash	-0.37 ± 0.09
P_2O_5 and N	0.84 ± 0.03
P_2O_5 and SiO_2*	-0.45 ± 0.08
P_2O_5 and ash	0.64 ± 0.06

* Represents percentages of ash rather than percentages of plant.

Significance of data

A book by ORR (46) contains an excellent summary of the data on the mineral content of pasture plants, therefore only data of particular interest are cited. In previous papers COOPER, *et. al.* (19, 20) have discussed some of the electro-chemical factors influencing the nutrition of organisms. It was pointed out that there is a correlation between the position of ions in the electromotive series and their activity in the soil collodial complexes. There is also a relation between the order of ion absorption by many organisms and the potential series. The strength of ions normally utilized by organisms may also affect the quality of radiant energy necessary for optimum growth.

An inspection of table I clearly indicates that the strong ions are absorbed in greater quantities than the weaker ones and that the metallic ash constituents are in a more or less definite order. This is in agreement with calculations made by the author from data on the ash analyses of

forty-eight plants reported by ROBINSON, *et. al.* (**52**). These show the order of constituents as follows: K_2O 34.36 per cent., CaO 19.63 per cent., MgO 7.13 per cent., Al_2O_3 1.34 per cent., Fe_2O_3 0.61 per cent., P_2O_5 9.87 per cent., SiO_2 9.86 per cent., SO_3 8.98 per cent., and Cl_2 5.49 per cent. The metallic elements are in the same order as their standard electrode potentials. ASTON (**5, 6, 7**) reports data on the constituents of 70 samples of pasture plants which include samples from regions of New Zealand where iron is deficient in forage plants. His average data for 47–70 samples are as follows: CaO 1.05 per cent., MgO 0.47 per cent., Al_2O_3 0.13 per cent., Mn_3O_4 0.045 per cent. and Fe_2O_3 0.041 per cent. ELVEHJEM and HART (**22**) reported 199 mg., 65.8 mg., and 13.5 mg. per kilogram of dry matter respectively for iron, manganese and copper in forty-seven feed analyses. All of these data suggest that many plants selectively absorb the strong ions or the elements with relatively high standard electrode potentials.

It is interesting to note that there is more Mn_3O_4 than Fe_2O_3 in the New Zealand forage plants. It was observed by RIGG and ASKEW (**51**) that on the poorer pastures the plants contained more manganese than iron, and were also characterized by their high content of silica. There does not seem to be a deficiency of iron in the soil but it is not absorbed by plants. Since the Mn ion is stronger than the Fe ion, manganese is apparently selectively absorbed and probably partially substituted for iron in the growth of the plant. The pasture plants grown on soils relatively high in available manganese may not contain enough iron for the maintenance of animals, since it is an active constituent of the blood. As iron is apparently not an active constituent of chlorophyll, elements which have similar physiological effects may partially substitute for it in the pasture plants.

Table I shows that potassium is the most abundant electropositive element in the ash of the plant analyzed. There is a relatively high percentage of K_2O in the ash of Kentucky blue grass and sweet vernal grass. It is lower in Rhode Island bent grass and poverty grass. The high K_2O content in the ash of Kentucky blue grass is in agreement with the response of this plant to potash fertilizers as reported by WHITE and HOLBEN (**67**) and WHITE and GARDNER (**68**). It is also noteworthy that there is 0.18 per cent. CaO in the ash of Kentucky blue grass and 0.33 per cent. CaO in Rhode Island bent grass. This is an interesting relationship because the Kentucky blue grass samples were taken mostly from limestone soils. The average pH value (6.79) indicates that the soils were well supplied with available calcium. Most of the Rhode Island bent grass samples were taken from non-limestone soils as is indicated by the average pH value of 5.18. These inverse relations between K_2O and CaO show that the ash constituents of plants are largely determined by the amounts of various constituents available in the soil.

The phosphorus and the nitrogen contents of Kentucky blue grass are also relatively high, which suggests that this plant requires a relatively high fertility level for its successful growth. Rhode Island bent grass is lower in phosphorus and nitrogen than Kentucky blue grass but it is higher in silica. These data suggest that Rhode Island bent grass will tolerate a lower fertility level than will Kentucky blue grass. The ash and nitrogen content decrease from Kentucky blue grass to poverty grass, which is a good measure of the relative tolerance of these plants to various soil fertility levels. The broomsedge and rice cut-grass are extremely high in silica and low in constituents which form strong ions. These two plants are very tolerant of shade. Since these plants utilize large amounts of weak electrolytes they should be capable of tolerating a low intensity and low quality of light.

Correlations between the various constituents of pasture grasses are given in table II. There is a negative correlation of -0.87 ± 0.023 between K_2O and CaO; a negative correlation of -0.44 ± 0.08 between CaO and P_2O_5; and a negative correlation of -0.45 ± 0.08 between the P_2O_5 and SiO_2. The correlation coefficients for the other constituents are positive or not significant. There is a high positive correlation between P_2O_5 and N. This might be expected in case either of these is a limiting factor in plant growth. These two nutrients are in the same family of elements; therefore, they would be expected to have similar functions in the nutrition of organisms. With certain legumes and where liberal amounts of available phosphorus and nitrogen are present a negative correlation may exist between the amounts of these materials in the plant. Legumes may often be relatively low in silicon and phosphorus since these plants have available to them nitrogen which forms relatively strong electro-negative combinations. ARCHIBALD and NELSON (2) present data showing that the phosphorus content of pasture plants was about 10 per cent. higher on unfertilized check plats than on plats liberally fertilized with a complete fertilizer and calcium nitrate.

Data presented by SCOTT (55) relating to the phosphorus deficiency in forage plants is of special interest in this connection. He found as an average of nine samples of alfalfa 2.39 per cent. CaO and 0.249 per cent. P_2O_5 or a ratio of Ca:P of 15.8:1 which is much wider than the ratios by which rickets have been produced experimentally in certain small animals. This suggests that plants can tolerate a wider range in the Ca:P ratio than do certain animals. The phosphorus content was not particularly low in the soil from which the alfalfa was taken but the element was not available to the plants. Where the Ca:P ratio is as wide as observed in these samples the phosphorus usually exists in stable combinations and its assimilation would probably require short wave lengths of light.

A negative correlation of -0.44 ± 0.08 was obtained between CaO and P_2O_5. Calculations made from the ash data of forty-eight plants published by ROBINSON, STEINKOENIG and MILLER (52) show a negative correlation of -0.51 ± 0.08 between CaO and P_2O_5. These data also show a negative correlation of -0.70 ± 0.055 between the sum K_2O, Na_2O and CaO, and the sum of SiO_2, P_2O_5, Cl_2 and SO_3. These negative correlations suggest that in soil relatively high in available strong basic materials the hydroxyl, bicarbonate, and carbonate ions may be absorbed by plants in relatively large amounts. The relatively weak hydroxyl and bicarbonate ions form compounds which are relatively unstable as compared to strong sulphate, phosphate and fluoride ions which form relatively stable compounds and require for optimum assimilation relatively short wave lengths of radiant energy.

The strong negative correlation between K_2O and CaO is in agreement with the finding of FONDER (24) on the K_2O and CaO content of alfalfa grown on various soils.

Some factors influencing the ash constituents of plants

Standard electrode potentials are quantitative values which are very useful in correlating data. Since the standard electrode potentials include the heat of solvation of ions, some of the low atomic weight elements, which have highly hydrated ions, may be out of the order of their replacement value. In case of the alkali metals the ionization potentials give a better ion series than the standard electrode potentials.

MICHAELIS (43) has shown that there is a close correlation between the order of ion adsorption by blood charcoal and the electromotive tension series; the stronger the ion the less the adsorption by blood charcoal.

Data on base exchange by GEDROIZ (25) and numerous others may be interpreted as showing that there is a correlation between the order of atomic ions in the electromotive series and their activity in the soil colloidal complexes. Data published by WILSON (70) and MATTSON (39) on the liberation of ions from soil colloids by electrodialysis are of particular interest in this connection. Their data may also be interpreted as showing that there is a close correlation between the order of elements in the potential series and the order of removal of cations from soil colloids by electrodialysis.

It is not probable that large amounts of atomic ions below calcium in the electromotive series with the exception of hydrogen will be found in the soil colloidal complex. The calcium ion is the lowest metallic atomic ion in the potential series which would be expected to exist in large amounts in soil colloidal complexes. The H-ion often ranks next to calcium in its activity in soil colloids. The activity of the H-ion in soil colloids is greater

than might be expected from its position in the potential series. The ionization potentials of elements in various stages of ionization presented by MILLIKAN and BOWEN (44) and NOYES and BECKMAN (45) suggest the explanation for the relative abundance of the H-ion in colloidal complexes of certain acid soils. The last normal valence electron in the nutrient elements below calcium in the potential series is more strongly bound than the single valence electron of hydrogen. Approximately 6.07 equivalent volts are required to remove the last bound electron in calcium, and 11.74 volts for the second valence electron of calcium. With magnesium, the next element below calcium in the potential series, the energy of removal for the first and second electrons is 7.56 and 14.98 equivalent volts respectively, whereas the energy required to remove the valence electron of hydrogen is approximately 13.5 equivalent volts.

Since the H-ion often ranks next to calcium in its activity in soil colloids large amounts of electropositive elements below calcium in the electromotive series would not be expected in the ash of plants. Since the ionization potential of hydrogen and magnesium are so near together it might be expected that approximately the same quanta of radiant energy would be required for their optimum assimilation. It is interesting to note that magnesium is essential for the formation of fats and oils. Where the soil colloids are relatively high in H-ions and Mg-ions the conditions are often favorable for the dominance of resinous plants such as coniferous and gum trees. Complex ions such as NH_4-ion may often be an important nutrient ion particularly on acid soils. It is apparently readily assimilated by certain plants.

Data on permeability have been assembled by STILES (63) and they suggest that there is a relationship between the order of absorption and the strength of ions. As previously noted, it should not be overlooked that the low atomic number elements, which may have highly hydrated ions such as lithium, in an ion absorption series may not be in agreement with standard electrode potentials.

Some of the most interesting and convincing data on the order of absorption of salts or ions are those reported by HOAGLAND and DAVIS (30), OSTERHOUT (48), and COOPER and BLINK (18). These very significant data may be interpreted to indicate that there is a very close relation between the strength of atomic ions and the absorption of electropositive elements. The data reported by COOPER and BLINK (18) suggest that certain organisms may absorb isosteric ions. *Halicystis* seems to selectively absorb neon-like cations while species of *Valonia* selectively absorb argon-like cations from sea water. Membranes may act as ionic or molecular sieves according to McBAIN and KISTER (40). Many organisms seem to be able to exclude some of the large strong ions, such as the xenon-like and

krypton-like cations. In considering the influence of dimensions of ions on the rate of penetration, the radii of hydrated as well as of unhydrated ions may be important. The conflicting, and probably much erroneous, data on permeability of ions make it very difficult to formulate a general statement concerning the order of penetration of materials. The best available data certainly suggest that many organisms tend to differentially absorb atomic ions in the same order as they appear in the electromotive series. Since the nature or porosity of membranes, the magnitude of the hydrated and unhydrated radii of ions, and the utilization of ions by organisms may greatly influence the order of absorption of materials, it is difficult to establish an absorption series for various membranes.

Relation of mineral content of plants to shade tolerance and feeding value

It is logical to assume (and the results from experiments seem to justify the assumption), that pasture plants grown on soils where the complex is favorable for autotrophic bacteria and the chemosynthetic production of carbohydrates, such as occurs in the nitrification and sulphofication processes, have a relatively high feeding value. Chemosynthesis is stimulated by the presence of metallic elements with relatively high standard electrode potentials such as K, Na and Ca. The free energy decrease in the formation of certain compounds of these elements is sufficiently large to satisfy the energy requirements of the autophytic soil organisms. BAAS-BECKING and PARKS (8) have summarized some of the data on the energy requirements of autophytic bacteria. BUCHANAN and FULMER (14) have also compiled data on the energy requirements of certain organisms. The utilization of the relatively stable nutrient compounds by crop plants will probably require relatively large quanta of light energy and may result in the formation of organic compounds of high feeding value. Plants which require a fertile soil, or strong ions, are not very tolerant of shade and usually have a high feeding value.

Since the discovery was made that light having a wave length between 3130 and 2900 Ångstroms or approximately 3.94 to 4.26 equivalent volts protect animals from rickets, there has been an interest in securing foods which may supply equivalent quanta of energy. The researches of STEENBOCK and BLACK (62), HESS and WEINSTOCK (29), KNUDSON and MOORE (36), LAURENS (37, 38) and numerous others have shown that the radiation which renders foods active biologically is similar in wave lengths to that which protects animals against rickets when they are directly exposed to the rays. SHOHL, BENNETT and WEED (58) and others have shown that the acid-base content of the diet is an important factor in either the production or the cure of experimentally induced rickets. These

an important factor in the catalytic activity of certain materials.   Therefore the energy necessary for the decomposition or the reduction of nutrients and the resonance radiation from activated materials are of special interest in nutritional studies.

It is possible to get the approximate relative minimum energy of decomposition of certain simple compounds from their standard electrode potentials (20).   The approximate relative energy of formation in volt-Faradays of various chlorides, calculated from standard electrode potentials and the equivalent quality of light energy, are given in table IV.   Radiant energy equivalent to or greater than the free-energy decrease in the formation of simple electrolytes may be necessary for their optimum assimilation by autophytic plants.   Elements which cannot be readily reduced by the radiant energy characteristic of the solar spectrum are not found as actual constituents of many organic compounds.

TABLE IV

APPROXIMATE RELATIVE HEAT OR ENERGY OF FORMATION, CALCULATED FROM ELECTRODE POTENTIALS, OF SOME NUTRIENT ELECTROLYTES AND THE CORRESPONDING EQUIVALENT ENERGY VALUE IN LIGHT

ELECTROLYTE	ENERGY OF FORMATION IN VOLT-FARADAYS	EQUIVALENT LIGHT ENERGY
$CuCl_2$	1.01	infra red
HCl	1.35	infra red
$NiCl_2$	1.57	infra red
$CoCl$	1.64	red
$FeCl_2$	1.78	red
NH_4Cl	1.90	orange
$CrCl_2$	1.95	orange
$ZnCl_2$	2.11	yellow
$MnCl_2$	2.45	green
$AlCl_3$	2.65	blue
$MgCl$	2.85	violet
$CaCl$	3.85	ultra-violet
$NaCl$	4.05	ultra-violet
KCl	4.28	ultra-violet
H_3PO_4	1.70*	red
$Ca(OH)_2$	2.91	violet
$Ca_2H_2(PO_4)_2$	4.20*	ultra-violet
CaF_2	4.46	ultra-violet

* Calculated from discharge potential of acid.

The relative equivalent energy of formation of $FeCl_2$ corresponds to the equivalent radiant energy in red light.   The corresponding value for $MgCl_2$

is equivalent to the energy of violet light. ARTHUR (3) has shown that the elimination of either the red or blue portion of the spectrum greatly affects the growth of certain plants. SHULL (59) has called attention to the selective absorption of light by leaves in the red and blue violet portion of the spectrum. It was observed by SAYRE (53) that wave lengths of radiant energy longer than 6800 Ångstroms, which is equal to 1.81 equivalent volts, are not effective in the formation of chlorophyll in seedlings. This value is very near the decomposition voltage of $FeCl_2$ which is 1.78 volts. The energy for the reduction of BO_3^{---} to BO_2^- is also 1.78 equivalent volts. This may indicate that certain iron and boron compounds selectively absorb similar quanta of radiant energy.

Chlorophyll "a" has a strong absorption band around 6800–6370 Ångstroms, equal to 1.87 to 1.84 equivalent volts. It has another strong absorption band around 4300–4400 Ångstroms, equal to 2.87 to 2.80 equivalent volts. These values are in close agreement with the decomposition voltage of $MgCl_2$ and the reduction of HCO_3^- to HCO_2^- which is 2.85 volts. This indicates that these two materials selectively absorb similar quanta of radiant energy. Some of the less intense absorption bands of chlorophyll are in close agreement with the decomposition voltages of certain compounds of elements absorbed by plants as shown in table IV. There is apparently selective absorption of radiant energy around the critical decomposition potential of certain nutrient materials. It seems as if growth of plants is intimately related to the absorption of radiant energy approximately equivalent to the decomposition or reduction energy of certain nutrient compounds. A paper on the relation of nutrients to the formation of chloroplast pigments by SCHERTZ (54) is of special interest in this connection. SPOEHR (61) has presented a comprehensive discussion of this general topic.

The relation of decomposition voltage to nutrition is nicely demonstrated in the prevention or curing of experimental rickets by ultraviolet radiation. Rickets induced by a high calcium and a low phosphorus diet can be cured by exposing animals to light wave lengths between 3130 and 2900 Ångstroms which is equal to 3.94 to 4.26 equivalent volts. Maximum activity is from 3020 to 2970 Ångstroms or 4.08 to 4.17 volts according to COBLENTZ and STAIR (16). The decomposition voltage of calcium phosphate as calculated from standard electrode potentials of Ca^{++} (2.50 volts) and the discharge potential of $H_2PO_4^-$ (1.70 volts) is 4.20 volt-Faradays, which is equivalent to the energy in ultraviolet light as shown in tables III and IV. This suggests that the prevention of rickets in animals utilizing calcium phosphate may require radiant energy approximately equivalent to the decomposition voltage of calcium phosphate. It is suggested by SHEAR,

WASHBURN and KRAMER (56) that bones may be composed of $CaHPO_4$ instead of $Ca_3(PO_4)_2$. CLARK (15) found that ultraviolet light decomposed compounds of calcium and protein, and increased the ionic calcium in blood serum. Food stuffs which supply quanta of energy necessary for decomposition of calcium compounds may also be effective in preventing rickets. The decomposition voltage for H_3PO_4 is 1.70 volts, which is equivalent to the energy in red light. The relative energy of formation of $Ca(OH)_2$ is 2.91 volts or equivalent to the energy in violet light. These data on the energy of formation of compounds suggest that rickets may in certain cases be prevented or cured by supplying the animal, either in food or in radiant energy, the quanta of energy equivalent to the decomposition voltage of the calcium and phosphorus compounds being utilized by the animal (56). The data presented by SHOHL, BENNETT and WEED (58) definitely suggest that where calcium and phosphorus are available in less stable combinations than calcium phosphate, relatively small quanta of radiant energy may produce normal development.

Calcium phosphate is light stable to near the extreme short wave length limit of the solar spectrum. It would not be expected to be light stable beyond the short wave length of the solar spectrum. The only other common nutrient calcium compounds which would be expected to be more light stable than calcium phosphate are compounds of calcium and fluorine as shown in tables III and IV. The enamel of teeth contains considerable fluorine which makes it very light stable, probably not being affected until the quanta of radiant energy are much larger than is characteristic of the solar spectrum. The relation of fluorine to the development of teeth has been discussed by McCOLLUM, SIMONDS and BECKER (42). Since considerable quantities of fluorine are found in many phosphate materials which are used as plant nutrients, fluorine may be present in many plants.

It is probable that many of the reactions caused by the absorption of radiant energy are due to oxidations and reductions or processes in which a change of valence takes place, with the liberation of O_2 from the reduced compound. The approximate equivalent minimum radiant energy required for the reduction of some common nutrient anions is given in table V (32). An inspection of this table shows that there is a great difference in the stability of the common nutrient anions. It is noted that the NO_3^- may be reduced by infra red, BO_3^{---} by red, HSO_4^- by blue, HCO_3^- by violet and $H_2PO_4^-$ by ultraviolet radiation. These data definitely suggest the reason why many plants respond so readily to nitrate nitrogen, particularly in nutrient solutions containing strong cations. The marked response of certain plants, particularly legumes, to boron compounds as reported by BRENCHLEY (12), SOMMER and LIPMAN (60), COLLINS (17), JOHNSTON and

DORE (33), may be definitely related to the small quanta of energy required for the reduction of borates. The sulphate and carbonate ions require a large quantum of energy for their reduction, while the phosphate ion is relatively stable and requires ultraviolet light for reduction. HARRIS (27) has noted that there is a group of light waves equal to 2.83–4.24 equivalent volts which has a marked effect upon the metabolism of rats and mice; an increase of 20 per cent. in CO_2 output was observed. These rays are very close to the minimum energy, 2.85 equivalent volts, necessary for the reduction of the HCO_3-ion which would probably result in the splitting off of O_2 and CO_2.

TABLE V

EQUIVALENT MINIMUM RADIANT ENERGY REQUIRED FOR THE REDUCTION OF SOME NUTRIENT ANIONS

NO_3^- + infra red radiation	11757 Å $= NO_2^-$ + 1/2 O_2
BO_3^{--} + red radiation	6935 Å $= BO_2^-$ + 1/2 O_2
HSO_4^- + blue radiation	4572 Å $= HSO_4^-$ + 1/2 O_2
HCO_3^- + violet radiation	4332 Å $= HCO_2^-$ + 1/2 O_2
$H_2PO_4^-$ + ultra-violet radiation	3685 Å $= H_2PO_3^-$ + 1/2 O_2
$H_2PO_4^-$ + ultra-violet radiation	3259 Å $= H_2PO_2^-$ + 1/2 O_2

The selective absorption of strong ions and of relatively stable compounds may limit or exclude the absorption of essential light sensitive elements, which have relatively low standard electrode potentials, such as iron, manganese and copper. The strength of ions which are endured by plants may influence their shade tolerance and palatability. In general the plants which grow well on relatively poor soil are more likely to be tolerant of shade, since they probably utilize mostly relatively weak ions. While the plants which require fertile soils and strong ions usually have a high feeding value.

The above mentioned relationships clearly indicate that there is a close relation between the heat, or energy, of formation and the energy required for decomposition, or the reduction of various nutrient compounds, and the quality of light necessary for their optimum assimilation by autophytic plants. The very interesting and significant work on the physiological effect of light at the Boyce Thompson Institute for Plant Research reported by ARTHUR (3), ARTHUR and NEWELL (4), POPP (49) and SHIRLEY (57) are of particular interest in this connection. Their work shows that plants respond differently to various intensities and qualities of light. SHIRLEY (57) found that redwoods and loblolly pine survive under a low light intensity. Also certain coniferous seedlings were found by BATES and ROESER (9) to tolerate very low light intensities. These relations might

be expected since such plants grow successfully on relatively poor soils and are tolerant of weak nutrients ions. But the sunflower plants which make their optimum growth on fertile soils and are very tolerant of strong ions require higher light intensity for optimum growth. The work of POPP (49) indicates that tomatoes and soybeans do not require for optimum growth as short wave lengths of light as do sunflowers and sudan grass.

The bimodal growth or production curve often observed in biological material may be related to the stability or sensitivity to solar radiation of the available nutrient compounds. Adsorption potentials and diffusion coefficients as calculated from mobilities of ions may also be important factors in determining bimodal growth curves.

Substitution of one nutrient element for another within a family or group of elements

The selective absorption of certain nutrient materials with relatively high standard electrode potentials indicates that within certain limits organisms may absorb materials with a positive or a negative charge of electricity rather than any specific ion. Therefore the ash constituents of a plant are largely determined by the available nutrients in the soil. This makes it necessary to consider groups of possible nutrient elements rather than any single ion. Since families of elements are qualitatively alike their chief differences are largely quantitative in character. Therefore one member of a family of elements may partially substitute for another in the nutrition of organisms. BREAZEALE (11) found that certain nutrients effect the absorption of others.

Elements may substitute for one another in nutrition in several ways (1) by substituting as an actual constituent of a specific organic compound, (2) in oxidation reduction reactions and (3) as catalytic agents. The possibilities of substitution in the two latter cases are probably much greater than in the first.

In the alkali metal family considerable quantities of Rb, K, Na and Li may be found in various plants. Certain plants can apparently utilize Rb for a certain quantity of K. More Rb than K was found in the growing points of cereals by RAMAGE (50). Certain plants seem to get along quite as well with Na as with K. A large amount of Li is toxic to many plants. There is often an inverse relation between the Mg, Ca, and Sr in organisms. Some relations between calcium and strontium in nutrition have been discussed by WHEELER (66), and KINNEY and McCOLLUM (35). HAAS (26) reports the inverse relation between the content of Ca and Mg in plants and their relation to chlorosis.

In the halogen family the elements differ widely in their electrical properties. Fluorine is the most electronegative element and its compounds are relatively light stable, while iodine forms unstable compounds. Iodine compounds are relatively easily decomposed by radiant energy which is characteristic of the solar spectrum. It is highly probable that the marked contrast in the physiological effect of F and I is related to their sensitivity to radiant energy. McClendon (41), and Orr and Leitch (47) have discussed extensively the relation of iodine to nutrition. Chlorine and bromine are intermediate between F and I in their properties, and there is apparently a rather wide range in the substitution of Br for Cl with certain organisms, according to data presented by Hoagland, Davis and Hibbard (31).

Nitrogen and phosphorus are in the same family of elements and they undoubtedly have many similar functions in the nutrition of organisms. A liberal quantity of one of these elements may enable an organism to function normally with a minimum quantity of the other. The works of Clark (15) and Csapo (21) suggest that under certain conditions a high blood protein content may interfere with the precipitation of calcium phosphate and normal bone formation. It is suggested that ultraviolet radiation may decompose the compounds of calcium and protein and increase the ionized Ca in the blood.

In oxidation-reduction reactions there is a possibility of a rather wide range of substitution. Nitrates, borates, sulphates, carbonates and phosphates may undergo reduction by the absorption of radiant energy characteristic of the solar spectrum.

Many of the elements which act as catalysts are found in the transition series in the periodic chart of the elements. The color of ions and the catalytic properties of elements in the transition series may be definitely related to the comparatively small energy difference between any two states in which valence electrons may exist. The resonance radiation from these easily activited elements may be an important factor in determining the catalytic activity of certain materials. Similar responses may be expected from elements with atomic numbers from 21 to 30 including Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu and Zn. It has been observed by Felix (23), Allison, Bryan and Hunter (1), Bryan (13), Willis (69), that certain of these constituents are effective in increasing plant growth, particularly on organic soils relatively high in strong cations. The researches of Hart, Steenbock, Waddell and Elvehjem (28), Titus, Cave, and Hughes (64) and Beard, Myers and Shipley (10) have shown that such materials as Mn, Fe and Cu may have similar or supplementary effects in the nutrition of animals. It is possible that similar physiological responses might be

produced by some of the transition elements in the fifth and sixth periods of the chart of the atoms.

Summary and conclusions

1. Ash analyses of pasture grasses, selected from the four major associations found in New York pastures, are presented.

2. These analyses show that there is a close correlation between the standard electrode potentials of elements and the amount of the various minerals in the ash of plants.

3. The samples taken from productive soils contained relatively large amounts of those elements which form strong ions, while the samples taken from unproductive soil contained a relatively low ash content and were relatively high in the elements which form weak ions.

4. A negative correlation exists between K_2O and CaO and between CaO an P_2O_5 in the ash of plants. Calcium seems to be negatively correlated with the strong anionic materials which suggest that plants growing on alkaline soils may absorb considerable quantities of the hydroxyl and bicarbonate ions.

5. There seems to be a relation between the strength of ions a plant will endure and its shade tolerance and feeding value. Plants which make their optimum growth on fertile soils and require strong ions are not very tolerant of shade and are of high feeding value, whereas plants which grow on poor soils and endure relatively large quantities of weak ions are more shade tolerant and usually have a lower feeding value.

6. Radiant energy may be effective in nutrition (1) by decomposing nutrient compounds, (2) by reducing or causing a change of valence of certain material and (3) by activating certain atoms or ions, which may emit resonance radiation. The approximate relative free energy decrease in the formation of certain simple electrolytes may be secured from standard electrode potentials.

7. The results of recent experiments by numerous investigators indicate that radiant energy equivalent to the decomposition voltage seems to greatly facilitate the assimilation of calcium phosphate. This is nicely demonstrated by the effect of ultraviolet light in the prevention or cure of rickets.

Since within certain limits organisms appear to absorb materials with a positive or negative charge rather than any specific ion, it is possible that one element may substitute for another within a family or group of elements. The extent of the possible substitution will probably vary with the organism and the properties of the elements involved.

Many catalytic agents are in the transition series of the periodic chart of the atoms. The color of ions and the catalytic properties of elements

in the transition series may be definitely related to the comparatively small energy differences between any two states in which valence electrons may exist. The resonance radiation from easily activated materials may be an important factor in determining the catalytic activity of certain materials. The energy relations discussed in this paper prove very useful in interpreting some of the recent experimental results on the effect of radiant energy on organisms.

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LITERATURE CITED

1. ALLISON, R. V., BRYAN, O. C., and HUNTER, J. H. The stimulation of plant response on the raw peat soils of the Florida Everglades through the use of copper sulphate and other chemicals. Florida Agr. Exp. Sta. Bull. 190. 1927.

2. ARCHIBALD, J. G., and NELSON, P. R. The chemical composition of grass from plots fertilized and grazed intensively. Jour. Amer. Soc. Agron. **21**: 686–699. 1929.

3. ARTHUR, J. M. Some effects of radiant energy on plants. Jour. Opt. Soc. Amer. **18**: 253–263. 1929.

4. ————————, and NEWELL, J. M. The killing of plant tissue and the inactivation of tobacco mosaic virus by ultra violet radiation. Amer. Jour. Bot. **16**: 338–353. 1929.

5. ASTON, B. C. Mineral content of pastures. New Zealand Jour. Agr. **36**: 22–27. 1928.

6. ————————. Mineral content of pastures. New Zealand Jour. Agr. **36**: 75–82. 1928.

7. ————————. Mineral content of pastures research. New Zealand Jour. Agr. **39**: 18–26. 1929.

8. BAAS-BECKING, L. G. M., and PARKS, G. S. Energy relations in the metabolism of autotrophic bacteria. Physiol. Rev. **7**: 85–106. 1927.

9. BATES, C. G., and ROESER, J. Light intensities required for growth of coniferous seedlings. Amer. Jour. Bot. **15**: 185–194. 1928.

10. BEARD, H. H., MYERS, V. C., and SHIPLEY, R. A. Blood regeneration in nutritional anemia. Influence of iron, iron and copper, nickel, cobalt, germanium or sodium germanate. Soc. Expt. Biol. and Med. Proc. **26**: 510–511. 1929.

11. BREAZEALE, J. F. The effect of one element of plant food upon the absorption by plants of another element. Arizona Agr. Exp. Sta. Techn. Bull. 19. 1928.

12. BRENCHLEY, W. E. Inorganic plant poisons and stimulants. Second edition, Cambridge University Press. 1927.

13. BRYAN, O. C. The stimulating effect of external applications of copper and manganese on certain chlorotic plants of the Florida Everglades soils. Jour. Amer. Soc. Agron. **21**: 923–933. 1929.

14. BUCHANAN, R. E., and FULMER, E. I. Physiology and biochemistry of bacteria. Williams & Wilkins Co. 1928.

15. CLARK, J. H. The effect of ultraviolet light on the condition of calcium in the blood. Amer. Jour. Hygiene **3**: 481–482. 1924.

16. COBLENTZ, W. W., and STAIR, R. Data on ultraviolet solar radiation and the solarization of window materials. Bur. Standards, Jour. Res. **3**: 629–689. 1929.

17. COLLINS, G. H. The influence of boron on the growth of the soybean plant. Soil Sci. **23**: 83–104 1927.

18. COOPER, W. C., and BLINKS, L. R. The cell sap of *Valonia* and *Halicystis*. Science n.s. **68**: 164–165. 1928.

19. COOPER, H. P., and WILSON, J. K. Correlation between electromotive series and oxidation potentials in plant and animal nutrition. Science n.s. **66**: 629–631. 1927.

20. ————, ————, and BARRON, J. H. Ecological factors determining the pasture flora in the northeastern United States. Jour. Amer. Soc. Agron. **21**: 607–627. 1929.

21. CSAPO, JOSEPH. The influence of proteins on solubility of calcium phosphate. Jour. Biol. Chem. **75**: 509–515. 1927.

22. ELVEHJEM, C. A., and HART, E. B. The copper content of feedingstuffs. Jour. Biol. Chem. **82**: 473–477. 1929.

23. FELIX, E. L. Correction of unproductive muck by the addition of copper. Phytopath. **17**: 49–50. 1927.

24. FONDER, J. F. Variations in potassium content of alfalfa due to stage of growth and soil type and the relationship of potassium and calcium in plants grown upon different soil types. Jour. Amer. Soc. Agron. **21**: 732–750. 1929.

25. GEDROIZ, K. K. On the absorptive power of soils. From a series of papers published in Zhur. Opytn. Agron. 1919–1923. (English translation from the Russian by S. A. WAKSMAN).

26. HAAS, A. R. C. Composition of avocado trees in relation to chlorosis and tip-burn. Bot. Gaz. **87**: 422–430. 1929.

27. HARRIS, D. T. Studies on the biological action of light. Proc. Roy. Soc. London B **98**: 171–187. 1925.

28. HART, E. B., STEENBOCK, H., WADDELL, J., and ELVEHJEM, C. A. Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. Jour. Biol. Chem. **77**: 797–812. 1928.

29. Hess, A. F., and Weinstock, M.   The antirachitic value of irradiated cholesterol and phytosterol.   II. Further evidence of change in biological activity.   Jour. Biol. Chem. **64**: 181–191.   1925.

30. Hoagland, D. R., and Davis, A. R.   The composition of the cell sap of the plant in relation to the absorption of ions.   Jour. Gen. Physiol. **5**: 629–646.   1923.

31. ——————, ——————, and Hibbard, P. L.   The influence of one ion on the accumulation of another by plant cells with special reference to experiments with *Nitella*.   Plant Physiol. **3**: 473–486.   1928.

32. International Critical Tables.   Volume **5**, 1929.   McGraw-Hill Co., New York.

33. Johnston, E. S., and Dore, W. H.   The influence of boron on the chemical composition and growth of the tomato plant.   Plant Physiol. **4**: 31–62.   1929.

34. Jones, M. R.   Hydrochloric acid therapy in rickets.   Jour. Amer. Med. Assoc. **82**: 439–440.   1924.

35. Kinney, E. M., and McCollum, E. V.   A study of the rate of deposition and paths of absorption of strontium in the rat.   Jour. Pharm. Exp. Ther. **21**: 165–176.   1923.

36. Knudson, A., and Moore, C. N.   Comparison of antirachitic potency of ergosterol irradiated by ultraviolet light and by exposure to cathode rays.   Jour. Biol. Chem. **81**: 49–64.   1929.

37. Laurens, H.   The physiological effects of radiation.   Physiol. Rev. **8**: 1–91.   1928.

38. ——————.   Some of the physiological effects of radiant energy.   Jour. Opt. Soc. Amer. **18**: 237–252.   1929.

39. Mattson, Sante.   Electrodialysis of the colloidal soil material and the exchangeable bases.   Jour. Agr. Res. **33**: 553–567.   1926.

40. McBain, J. W., and Kistler, S. S.   Membranes for ultrafiltration of graduated fineness down to molecular sieves.   Jour. Gen. Phys. **12**: 187–200.   1928.

41. McClendon, J. F.   The distribution of iodine with special reference to goiter.   Physiol. Rev. **7**: 189–258.   1927.

42. McCollum, E. V., Simmonds, N., Becker, J. E., and Bunting, R. W.   The effect of additions of fluorine to the diet of the rat on the quality of the teeth.   Jour. Biol. Chem. **63**: 553–562.   1925.

43. Michaelis, L.   Hydrogen-ion concentration.   Williams & Wilkins Co., Baltimore.   1926.

44. Millikan, R. A., and Bowen, I. S.   Energy relationships and ionization potentials of atoms of the first row of the periodic table in all stages of ionization.   Proc. Nat. Acad. Sci. **13**: 531–535.   1927.

45. NOYES, A. A., and BECKMAN, A. O. A periodic table of the structure of atoms and its relation to ion formation and valence. Proc. Nat. Acad. Sci. **13**: 737–743. 1927.

46. ORR, J. B. Minerals in pastures and their relation to animal nutrition. H. K. Lewis & Co., Ltd. London. 1929.

47. ——————, and LEITCH, I. Iodine in nutrition. A review of existing information up to 1927. (Gr. Brit.). Med. Research Council, Spec. Rpt. Ser.. no. **123**. 1929.

48. OSTERHOUT, W. J. V. Some aspects of selective absorption. Jour. Gen. Physiol. **5**: 225–230. 1922–1923.

49. POPP, H. W. A physiological study of the effect of light of various ranges of wave length on the growth of plants. Amer. Jour. Bot. **13**: 706–736. 1926.

50. RAMAGE, HUGH. Spectrographic chemical analysis. Nature **123**: 601–602. 1929.

51. RIGG, T., and ASKEW, H. O. Mineral content of some typical pastures in Waimea County. New Zealand Jour. Agr. **38**: 304–316. 1929.

52. ROBINSON, W. O., STEINKOENIG, L. A., and MILLER, C. F. The relation of some of the rarer elements in soils and plants. U. S. Dept. Agr. Bull. 600. 1917.

53. SAYRE, J. D. The development of chlorophyll in seedlings in different ranges of wave lengths of light. Plant Physiol. **3**: 71–77. 1928.

54. SCHERTZ, F. M. The effect of potassium, nitrogen and phosphorus fertilizing upon the chloroplast pigments, upon the mineral content of the leaves and upon production in crop plants. Plant Physiol. **4**: 269–279. 1929.

55. SCOTT, S. G. Phosphorus deficiency in forage feeds of range cattle. Jour. Agr. Res. **38**: 113–130. 1929.

56. SHEAR, M. J., WASHBURN, M., and KRAMER, B. Composition of bone. VII. Equilibration of serum solutions with dicalcium phosphate. Jour. Biol. Chem. **83**: 697–720. 1929.

57. SHIRLEY, H. L. The influence of light intensity and light quality upon the growth of plants. Amer. Jour. Bot. **16**: 354–390. 1929.

58. SHOHL, A. T., BENNETT, H. B., and WEED, K. L. Rickets in rats. IV. The effect of varying the acid-base content of the diet. Jour. Biol. Chem. **78**: 181–190. 1928.

59. SHULL, C. A. A spectrophotometric study of reflection of light from leaf surfaces. Bot. Gaz. **87**: 583–607. 1929.

60. SOMMER, A. L., and LIPMAN, C. B. Evidence on the indispensable nature of zinc and boron for higher green plants. Plant Physiol. **1**: 231–249. 1926.

61. SPOEHR, H. A. Photosynthesis. Chemical Catalog Co. New York. 1926.

62. STEENBOCK, H., and BLACK, A. Fat soluble vitamins. XXIII. The induction of growth-promoting and calcifying properties in fats and their unsaponifiable constituents by exposure to light. Jour. Biol. Chem. 64: 263–298. 1925.

63. STILES, WALTER. Permeability. Wheldon & Wesley, Ltd. London. 1924.

64. TITUS, R. W., CAVE, H. W., and HUGHES, J. S. The manganese-copper-iron complex as a factor in hemoglobin building. Jour. Biol. Chem. 80: 565–570. 1928.

65. TOTTINGHAM, W. E., and LOWSMA, H. Effects of light upon nitrate assimilation in wheat. Jour. Amer. Chem. Soc. 50: 2436–2445. 1928.

66. WHEELER, W. P. Studies relating to calcium metabolism. New York Agr. Exp. Sta. Bull. 468. 1919.

67. WHITE, J. W., and HOLBEN, F. J. Development and value of Kentucky blue grass pastures. Pennsylvania Agr. Exp. Sta. Bull. 195. 1925.

68. ——————, and GARDNER, F. D. Soil fertility experiments on Volusia and Westmoreland soils. Pennsylvania Exp. Sta. Bull. 229. 1928.

69. WILLIS, L. G. Response of oats and soybeans to manganese on some Coastal Plain soils. North Carolina Agr. Exp. Sta. Bull. 257. 1928.

70. WILSON, B. D. Extraction of adsorbed cations from soil by electrodialysis. Soil Sci. 28: 411–421. 1929.

PRELIMINARY RESULTS IN MEASURING THE HARDINESS OF PLANTS[1]

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(WITH TWO FIGURES)

The injury of plants occasioned by winter conditions has been a subject of great interest and importance to plant breeders and agriculturists. The development of hardy strains has been, and is, one of the principal means of combating losses caused by injury from cold, although cultural methods, as well, have been adapted to help the plant survive the rigors of its climatic environment.

Recognition of the degree of hardiness has necessitated field trials commonly requiring extended periods of time, during which weather conditions may be too mild to test the relative hardiness in one season, while so severe in the next that hardy as well as tender varieties and strains are severely injured. It is quite evident that a rapid means of measuring relative degrees of resistance to cold of strains and varieties of plants, grown with various cultural procedures, would hasten progress in the work of plant breeders, as well as facilitate investigations of the physical and chemical nature of the hardening process, and its relation to the resistance of plants to low temperatures and other climatic conditions.

No attempt will be made to review the literature on hardiness or reactions of plants to freezing in reference to changes in permeability, colloidal structure or compositional variations. It seems to be generally accepted that the injury, or killing of tissue, by cold, or by any other means, involves the disorganization of the substances essential for carrying on the processes of life. With such disorganization, it is well recognized that the cell loses its capacity to regulate the diffusion of its soluble contents. Upon this basis, it was assumed that the degree of injury from low temperature, to overwintering and other plant structures might be correlated with the exosmosis of electrolytes and other materials following exposure to cold. Such outward diffusion of electrolytes can readily be estimated by conductivity measurements. Other investigators have applied conductivity methods to problems involving the viability of seeds, and the injury of plant and animal tissues.

To test this hypothesis, alfalfa roots of three varieties of known hardiness (Grimm, Utah Common, and Hairy Peruvian) were frozen under con-

trolled conditions, for various lengths of time, and at various stages of growth throughout the autumnal season. The plants used were grown under fertile soil conditions from seed sown on July 13, 1928, in plats on the University Farm at Madison, Wisconsin. They were not cut that season, and the stands in all plats were excellent. They survived the very favorable winter season of 1928–29 without apparent injury.

During the growing season of 1929, a half of each plat was cut twice, on July 9 and September 12, when the plants were in full bloom, while the remaining half of each plat was cut four times, on June 22, July 9, August 13, and September 12. This latter treatment was one which would greatly inhibit the storage of organic foods in the roots, while the two-cutting treatment would provide for an abundance of such reserves.

Preliminary work during the early autumn showed that much more extensive outward diffusion into distilled water occurred when the roots had been frozen than when they were uninjured, but no varietal differences were observed. On September 21, 1929, a standardized procedure was first applied to this study. Roots were dug from the plats of alfalfa cut only twice and such alfalfa was therefore "high" in reserve food content. They were trimmed at the crown, to leave buds for further development, cut to a uniform length of seven inches, then washed hurriedly but thoroughly in running water, by taking a small bunch of roots and rubbing them between the hands, thus giving little opportunity for exosmosis in the washing process. The roots in bunches of several hundred were then covered with a damp cheese cloth, whereupon gradual evaporation brought the specimens to a uniform condition of surface moisture.

Samples consisting of 8 roots, trimmed at the base when necessary, to weigh 20 grams, were selected and placed in Pyrex test tubes (1 x 8 in.), closed with rubber stoppers. Five such tubes of each variety were placed in a circular rack, for each freezing treatment. The freezing was carried on in a cylindrical ice cream can of five gallon capacity, in a large thermostatically regulated electrical refrigerator, where temperatures ranged between $-8°$ C. and $-9°$ C. The roots were left at this temperature for 1.5 hours, 4 hours, and 15 hours. In each case, five tubes of roots of each variety were similarly prepared, but not frozen. Thus, 15 tubes were not frozen but were used as checks; 15 were frozen for 1.5 hours; 15 for four hours; and 15 for 15 hours. Each tube contained 8 roots, weighing 20 grams. After freezing, the roots remained in the tubes at room temperature for several hours. Three tubes of each variety from each freezing treatment were selected for transplanting in the greenhouse, where their recovery and subsequent growth might be observed. The two remaining tubes of each variety, from each freezing treatment, were placed in a rack.

This rack was suspended in a thermostatic water-bath, at 25° C., and to each tube, in succession at 2.5 minute intervals, 50 cc. of distilled water was added from a pipette. This water completely covered the roots. The tubes of roots remained in the bath for a ten-hour period.

At the expiration of this time, the liquid about the roots was withdrawn from each tube in succession, with a pipette, redischarged into the tube for mixing, and then again withdrawn and transferred to a conductivity cell, where the reading of resistance was taken to the nearest ohm. This precaution of thorough mixing appeared, upon investigation, to be unnecessary, but was nevertheless adhered to. The roots were removed from the tube and the solution was preserved for further chemical study. In this way, each tube of roots had an equal time for exosmosis into water. In several preliminary experiments it had been found that there was little change in conductivity after ten hours, and this interval was, therefore, chosen for all later experiments.

The freezing periods of 1.5 hours, 4 hours and 15 hours were chosen after some study, in the hope that the brief freezing would show slight injury, while the fifteen-hour freeze would completely kill all varieties. The one and one-half hour freeze was discontinued after four trials because the injury to the roots, as shown by the greenhouse checks, was slight in the later part of autumn. The fifteen-hour freeze was sometimes omitted to permit other experiments to be run. The four-hour freeze was run regularly at weekly intervals.

The average values for the specific conductivity, determined in duplicate in each case, and expressed in reciprocal ohms, on each date, for each variety are given in table I, and are represented graphically in figure 1. The figures presented are for the four-hour freeze.

TABLE I

SPECIFIC CONDUCTIVITIES ($\times 10^4$) IN RECIPROCAL OHMS, OF WATER EXTRACTS FROM ROOTS
OF ALFALFA, AFTER FREEZING FOR FOUR HOURS. AN INTERVAL OF TEN
HOURS WAS ALLOWED FOR EXOSMOSIS

VARIETY	SEPT. 21	OCT. 11	OCT. 18	OCT. 25	NOV. 1	NOV. 8	NOV. 15	NOV. 27
Grimm	1632	1250	1015	921	549	781	629	484
Utah Common	1657	1255	1235	1200	1177	1215	1270	879
Hairy Peruvian	1624	1459	1375	1535	1365·	1447	1257	1475

No varietal difference was found in hardiness as measured by exosmosis from samples dug and frozen September 21, but during the latter part of October and the month of November prominent varietal differences are evi-

dent. This indicates that a very definite hardening process occurs in Grimm alfalfa during the fall period, while Peruvian has about the same resistance to cold on November 27 as on September 21. The Utah Common is intermediate throughout this period, but the hardening process seems to be delayed until the advent of freezing weather on or about November 19.

Apparently, the reaction of the plant to the climatic environment of the autumn period has much to do with its resistance to cold. The external appearance of these varieties growing under field conditions was markedly different during October and November. The last cutting was made on September 12, but owing to dry weather, there was very little growth recovery in any variety until September 28, when regeneration was stimulated by abundant rainfall. The character of this recovery varied widely in the different varieties. The growth of the Peruvian was rapid and up-
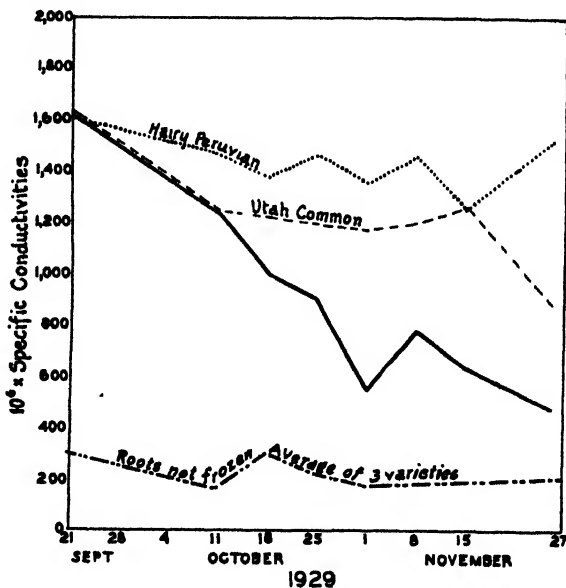
FIG. 1. Specific conductivity of water extracts from roots of alfalfa after freezing for four hours, and with an interval for exosmosis of ten hours. (Solid curve represents Grimm).

right, and on November 27 the frozen top growth measured about 14 inches. The Grimm alfalfa grew very slowly, and instead of being upright, was semi-decumbent. Just prior to freezing weather, the stems averaged about three or four inches in length. The Utah Common alfalfa was somewhat intermediate between the Grimm and the Peruvian with respect to its top growth. This description of the autumnal development of the three varieties is but another way of expressing the differences in dormancy.

No killing frost occurred until late in the season, and the top growth of the Peruvian was not killed until November 19, while the ground remained unfrozen until November 21. On November 27, when the last samples were dug for the conductivity experiments, the ground was frozen to a depth of about five inches. The exosmosis of all such samples tested without further freezing showed no injury from this frozen condition of the soil, while additional artificial freezing for four and fifteen hours (figures 1 and 2) caused considerable damage to the roots. The outward
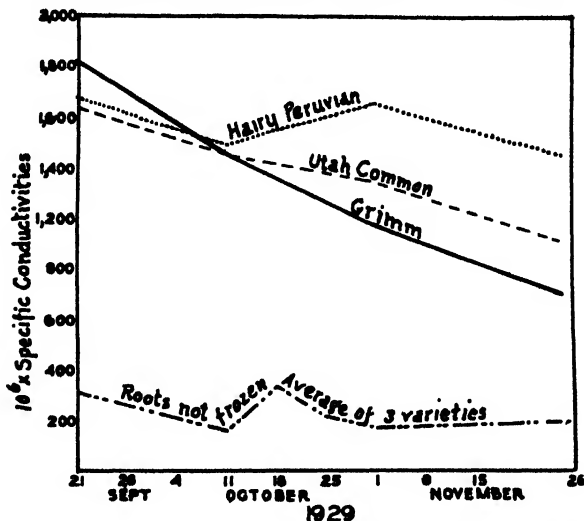
FIG. 2. Specific conductivity of water extracts from roots of alfalfa after freezing for fifteen hours and with an interval for exosmosis of ten hours.

diffusion of electrolytes is also indicated in table II by conductivity measurements after a fifteen-hour freezing treatment. Each value is the average of two determinations. This table is expressed graphically in figure 2. Again it is evident that Grimm alfalfa was no more resistant to cold on September 21 than were the other varieties, but in November, prominent differences are evident.

TABLE II

SPECIFIC CONDUCTIVITIES ($\times 10^6$) IN RECIPROCAL OHMS OF WATER EXTRACTS FROM ROOTS OF ALFALFA FROZEN FIFTEEN HOURS. AN INTERNAL OF TEN HOURS WAS ALLOWED FOR EXOSMOSIS

VARIETY	SEPT. 21	OCT. 11	NOV. 1	NOV. 27
Grimm	1820	1457	1191	696
Utah Common	1647	1463	1357	902
Hairy Peruvian	1680	1499	1689	1572

In order to check the method against another well established variation in hardiness, an experiment was carried out on November 8 and November 15 with alfalfa plants dug from the half of each plat of alfalfa cut four times, so as to facilitate a comparison with the plants cut only twice. With each variety, and in every case, without exception, the roots of alfalfa with low organic food reserves, due to four cuttings, gave a higher value for the specific conductivity than the corresponding roots with high reserves, from plants cut only twice. It has been shown that frequent cuttings lessen the hardiness of alfalfa and such results are corroborated by the conductivity measurements. Table III gives the averages of the two pairs of determinations, which checked very closely, and which were made on roots dug on November 8 and November 15. Differences between Grimm alfalfa .with high and low reserves are pronounced, as are also the differences in the Hairy Peruvian with reference to reserves. The Utah Common did not show such wide differences in this respect. In accordance with the results of other investigators, the roots of the several varieties as sampled

TABLE III

AVERAGE SPECIFIC CONDUCTIVITIES OF THE EXTRACTS FROM ALFALFA ROOTS HIGH AND LOW IN RESERVE FOODS. PLANTS WERE DUG ON NOVEMBER 8 AND 15, AND THE ROOTS WERE GIVEN A FOUR-HOUR FREEZING TREATMENT. AN INTERVAL OF TEN HOURS WAS ALLOWED FOR EXOSMOSIS

VARIETY	CUTTING TREATMENTS	
	TWO CUTTINGS	FOUR CUTTINGS
Grimm	705	1145
Utah Common	1243	1346
Hairy Peruvian	1302	2093

after the four-cutting treatment showed a significantly lower percentage of dry matter than those receiving the two cutting treatment. In percentage dry matter as well as in conductivity, the Utah Common, however, showed distinctly less difference between the roots with high and low reserves than the other varieties.

As another means of distinguishing differences in the concentration of the exudates in the exosmosis test, the dipping refractometer was used, and was found very useful in detecting the larger variations. The instrument is not as sensitive as the conductivity apparatus and the slight turbidity of the solution often made the readings uncertain when small differences might have existed. No attempts were made to clear the solutions before examining them. The turbidities of these solutions, however, gave evidence of the degree to which exosmosis had proceeded, and very commonly the

greater turbidity of the solution from the Peruvian distinguished it from the Utah Common, which in turn was more turbid than that of the Grimm variety. Preliminary experiments indicate that the percentage of solids in the solutions as determined by simple evaporation may be definitely correlated with the conductivity figures. Hydrogen-ion concentration, determined on only one of the experimental dates, October 18, gave no definite correlation with exosmosis and freezing injury. The volume of the precipitate with lead acetate appeared, superficially to be about the same for all varieties.

The concentration of sugars in the solution was also followed throughout most of the season, but no fixed relationship appeared to hold. In general, the Utah Common showed greater exosmosis of sugars than the others, and Grimm showed the least.

In order to correlate the amount of injury from each freezing treatment with subsequent growth, twenty-four plants of each variety were transplanted in soil under greenhouse conditions. In general, the development of these plants gave definite indications of a distinct correlation between high conductivity and freezing injury. It is difficult, however, to express the degree of growth response in specific terms. While the growth recovery after freezing was greatest with the Grimm and always the least with the Peruvian, there were wide variations in different plants of the same variety. Thus, some roots were severely injured at the lower end, but the crown survived sufficiently to send out shoots, while others were injured at the crown and entirely failed to give growth, although the bulk of the root appeared to be relatively sound. Still other roots failed to regenerate even though they seemed to be very slightly injured. The renewal of growth by the plants subjected to the freezing treatments is often very slow, especially when they have been severely injured. In some cases plants were rejected as dead after a period of several weeks, whereas growth finally began after further opportunity. Roots usually die, however, if growth does not occur within three or four weeks, and late growth is always sparse. It is, therefore, difficult at this time to present concise data on the regeneration of growth in the greenhouse, after the freezing treatment. In every case the Peruvian, frozen one and one-half hours, was markedly slower in its early growth recovery than the Utah Common and the Grimm. In no case, did plants other than Grimm survive the four-hour freeze. It seems likely that the maturity of the buds at the crown may be an important factor in regeneration after freezing.

As a further aid in the determination of relative hardiness by the principle of exosmosis from injured tissue, the following colorimetric tests have been tentatively tried on some series, with very promising results. It

must be emphasized that the values of determinations of a given ion need not necessarily be in the same ratio as the conductivity values. The organic materials as well may interfere with the tests. However, in this experiment, tests for the chloride ion appeared to correlate very well with the conductivity measurements, as far as they have been carried out. Such tests may well supplant the conductivity measurement in some cases, and in others, they may supplement it.

To compare the concentrations of the chloride ion in the water extract of the roots after freezing, 10 cc. of each solution was treated with 1 cc. of 5 per cent. potassium chromate solution and 15 drops of N/100 silver nitrate. In the absence of chlorides, the solution turns a deep red, or in other terms, presence of chlorides in the extract inhibits the development of the red color of silver chromate. If there is sufficient chloride to precipitate all of the silver, the original yellow color will remain. Solutions from roots of Hairy Peruvian alfalfa, frozen 4 or 15 hours, show much less coloration of red silver chromate than solutions from roots of Utah Common, and in turn, Utah Common show less than Grimm. Thus, Peruvian has given more chloride to the solution, by exosmosis, than the other varieties. The test when applied to the extract from unfrozen roots shows practically negligible exudation of chlorides, and the solution turns a deep red on the addition of the silver nitrate. The colors produced in the solutions remain unchanged for several hours and may be referred to a series of standard chloride solutions, similarly treated for quantitative comparison from week to week.

Organic substances interfered with the tests for nitrates with ferrous sulphate and sulphuric acid, but the colorations produced in 50 per cent. sulphuric acid appeared to correlate fairly well with the conductivity measurements.

These methods should be further standardized. Tests made for the calcium and sulphate ions were not sufficiently definite to appear of value and will not be described here.

A considerable number of possible variables have been investigated in this preliminary work. The size of the roots appears to make no difference in either cold injury or exosmosis, within the limits of our field conditions. Small roots may be cut longer than usual, or thick roots may be cut to one-third of ordinary length to make up the twenty grams of tissue. It does not appear to be necessary to use a given number of roots, so long as a given weight is employed. The roots may be cut off below the crown without affecting the experiment appreciably, but if the crown tissue is cut away, such roots are not suitable for greenhouse tests of growth recovery. Total ash determinations on the roots throughout the fall do not show

marked differences in the three varieties. The influence of soil added to the solution in considerable quantities was determined, and does not appear to be one of the larger variables. The rate of thawing may have considerable influence on the amount of injury and the amount of diffusion, but the influence of this variable was not measured. The rate of thawing, however, was quite uniform in all the trials reported in this paper.

The common precautions in conductivity work were employed. The tubes for exosmosis and their stoppers were soaked in distilled water for a day or two before use, and contamination by electrolytes was carefully avoided. With ordinarily careful technique, it does not appear that the unavoidable errors in the conduct of the measurements described are sufficient to affect, in a large degree, the accuracy of these determinations.

There are many incompleted problems left for further study, which will occur to the reader. The possible applications of the method to plants other than alfalfa is obvious. The writers plan to investigate some of these during the coming months, and to further establish the relationships that other variables may have in the reliability of the methods as outlined. It seems very likely that modifications, particularly of the colorimetric tests will be desirable, at least, in applying them to other plants.

Summary

A series of experiments and data has been presented indicating that the degree of resistance of plants to injury by cold weather may be measured by the diffusion of electrolytes and other substances from chilled or frozen tissues into water after such tissues have thawed. The amount of diffusion has been determined with alfalfa roots by conductivity measurements, which have been supplemented by colorimetric tests for chlorides. Within the limits of this investigation there exist correlations between known hardiness of alfalfa roots and the degree of retention of electrolytes by the tissue after freezing.

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DORMANCY AND GROWTH STUDIES OF THE AMERICAN LOTUS, *NELUMBO LUTEA*

WILLIAM C. MEYER

(WITH EIGHT FIGURES)

Attempts to establish the beautiful native American lotus in new locations have often been marked by failure, a fact which evokes scientific interest in the growth and dormancy habits of this plant. In spite of the fact that embryos have been known to sprout after two hundred years of dormany (6), it is doubtful if the plant reproduces from seed at all frequently in nature. The facile multiplication of tubers and the rapid growth of shoots from these create a competition for light and space which seedlings, if started under natural conditions, could probably not overcome. With the lotus in nature primarily dependent upon tubers for perpetuation, it is not surprising that florists in commercial production also rely chiefly upon them rather than upon seeds. The tubers produce a large flowering plant in a single season while the seedlings require at least two and sometimes probably as many as four years for floral development.

No record has been found in the literature of successfully growing the plant from seed. It is stated (3) that if holes are made in the fruits and they are then placed in a pond, their embryos are so delicate that they do not survive. In another case no signs of plants were found a few years subsequent to the planting of thousands of the fruits sown just as they came from the pods (3). The writer is personally acquainted with men who have had similar failures.

Scientifically, interest has centered largely in the germination of Nelumbo seeds. Old Indian lotus fruits obtained by OHGA from a prehistoric peat bed in South Manchuria are the most striking examples of protracted dormancy known to science (6). These were estimated to be three hundred to four hundred years old and certainly not less than two hundred years. A long period of dormancy has not been recorded for the American species but the fruit structure of the two species is practically alike and no sign of swelling occurs in either after being kept in water for several months (2).

OHGA (6) found concentrated sulphuric acid treatment to be the easiest method of overcoming this dormancy. Fifty per cent. chromic acid and concentrated potassium hydrate also overcome the impermeability after a longer period of time. According to his work, practically all of the fruit coat shows the cellulose reaction. SHAW (9) describes the cells as having a middle lamella of a mixture of pectic compounds and lignin, and a cellu-

lose lamella on either side which becomes very thick in the palisade cells composing the impermeable layer. She also describes a deposit of suberin in a lamella between the cellulose and the middle lamella of the palisade. In the cells bordering the stomatal cavities the suberin would be the material exposed to reagents. In her conclusions she states that "the only possible way to cause water to enter these stomatal cavities in *Nelumbo lutea* is to remove the fatty suberin lamella by some solvent. Aside from this method the only way to germinate these fruits is by filing the coats through the palisade layer, or by removing this layer by strong reagents."

Both OHGA and SHAW considered the stomatal cavities as places of attack but OHGA also considered the vascular scar at the basal end, the protuberance and the stylar canal, as points of water entry. After a thorough microscopical investigation he suggested a possibility of entrance at the stylar canal but said it would be rather difficult, and thought that it would be impossible at the other two points. In the present work, treatment with Schweitzer's reagent for seventy-five to two hundred hours made the fruits permeable, and the embryos developed normally. A three-hundred hour treatment destroyed the embryos. The reagent apparently gained entrance at the stylar end, that being the first portion of the coat to show swelling after the fruits were placed in water. SHAW probably is correct in stating that a fat solvent is necessary for dissolving the retarding layer in the stomatal cavities, but the statement that the only possible way to cause water to enter is by means of such a fat solvent, filing, or strong reagents does not appear to be correct, unless Schweitzer's reagent be classed as one of the latter. Further, in view of the specificity of Schweitzer's reagent, cellulose tissue, doubtless, participates in maintaining the impermeability of the fruit coats to water. Thus, a cellulose layer of cells may be chiefly responsible for the protracted dormancy of Nelumbo fruits.

Despite the searching investigations of OHGA (4, 5, 6, 7), SHAW (9), JONES (2) and others on the structure and behavior of the fruits, little is known concerning the development of seedlings or methods of establishing the plant in new locations by means of fruits or tubers. Difficulty in establishing new lotus beds suggests that the normal development of the plant may be profoundly influenced by certain specific environmental factors. This report deals primarily with the effects of certain factors on the growth of *Nelumbo lutea*.

Methods

The physico-chemical nature of the soil, as well as temperature, were thought to have some influence on the development and distribution of the plant. The northern limit of distribution is at Lake Pepin in the Mississippi River between Minnesota and Wisconsin. The low temperature of northern streams and lakes may prohibit further advance northward.

Higher temperatures from 20° to 30° C. have been found to greatly accelerate growth, while below 15° C. growth is very limited. This was partly determined by growing both seedling and large-tuber plants at 10°, 15°, 18° and 25° C. The accompanying photograph of seedlings (fig. 1) clearly
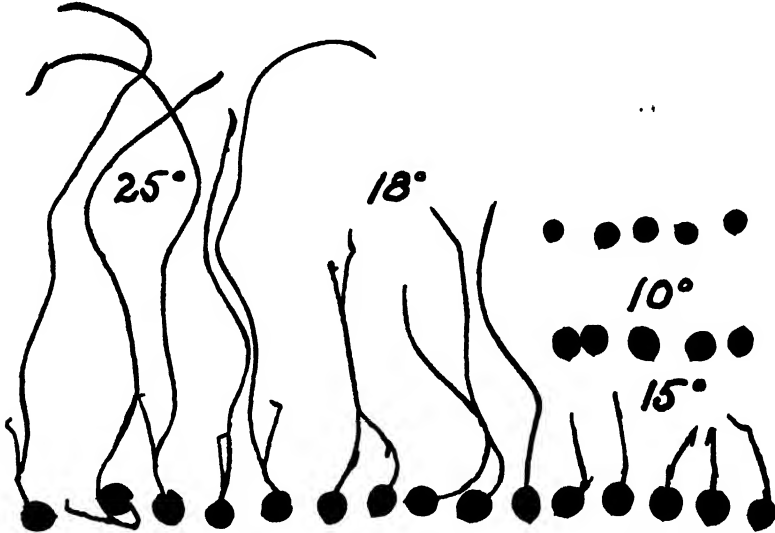
FIG. 1. Growth of seedlings in tap water at different temperatures. Fruits at upper right not treated to break impermeability.

shows that growth is directly related to temperature. The higher the temperature the more rapid the growth for the temperatures used. The upper thermal limit at which the plant will grow was not determined.

Slow growth, due to low temperature, would not permit successful competition with vigorous northern plants; but this does not explain the inability to establish the plant in new locations of similar temperature. The northward advance may also be prevented by the depth of winter frost. That the winter-tubers are destroyed by freezing was readily determined by freezing the tubers in water, at − 2.0° to − 2.6° C., for two hours. BISSET (1) and TRICKER (10), authorities on water gardening, both state that the tubers are destroyed by freezing. Destruction of the tubers in this manner may account for the lack of success in a new location of shallow water. But the depth of ice rarely exceeds two feet even in our northern lakes. As the tubers are located six to twenty inches in the soil beneath the water it appears that other factors are involved in nature.

Hydrion concentration has been found to be very influential in the development and distribution of plants (11, 12, 13), but little is known of its effect on the germination and development of aquatic spermatophytes. The large American lotus proved excellent material for such observations.

For a study of the effect of various hydrion concentrations galvanized iron tanks, 60 x 60 x 120 cm., painted within with an impermeable asphaltum paint, were used (fig. 2). Additions of sulphuric acid and sodium
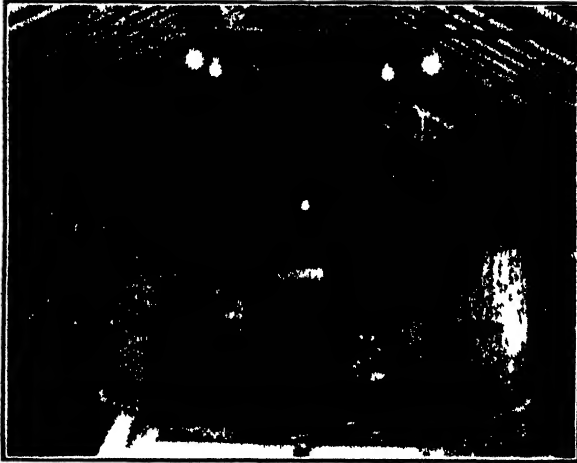
FIG. 2. Photograph of equipment for the study of effect of pH on seedling development. Connections for aëration and lighting are shown.

hydroxide gave the desired pH values. The continued shifting toward the neutral point by both acid and alkali solutions was corrected by regular additions of small amounts of normal solutions. Daily colorimetric tests were made and checked potentiometrically at intervals.

After the experiment had been running a month and a half, air was bubbled into the water through blocks of tulip poplar to maintain circulation and to prevent the growth of surface organisms. Electric light was used in addition to the daylight during the winter months (fig. 2), a one-hundred watt bulb being placed above each tank. The lights were turned off during the normal period of darkness. Plants were also grown in a cement basin (painted inside with the asphaltum paint to avoid rise in alkalinity). A small stream of tap water was allowed to run continually through the tank, resulting in a pH of 7.7.

Data and discussion

The plants grown in the basin showed a normal development as evidenced by their similarity to plants grown under natural conditions the previous year in a preliminary study. Four leaves form and then the rhizome. There is no hypocotyl and roots do not appear below the cotyledons but at the bases of the true leaves (fig. 3). The second internode of the rhizome becomes enlarged into a tuber (figs. 3 and 4) and the plant

Fig 3   Photograph of typical seedling plant at age of two weeks showing the four peltate leaves which were present in embryonic form within the one seeded fruit (remains of which are still attached), roots from the bases of these leaves and first node of rhizome, and the tuber developed in the second internode of the rhizome
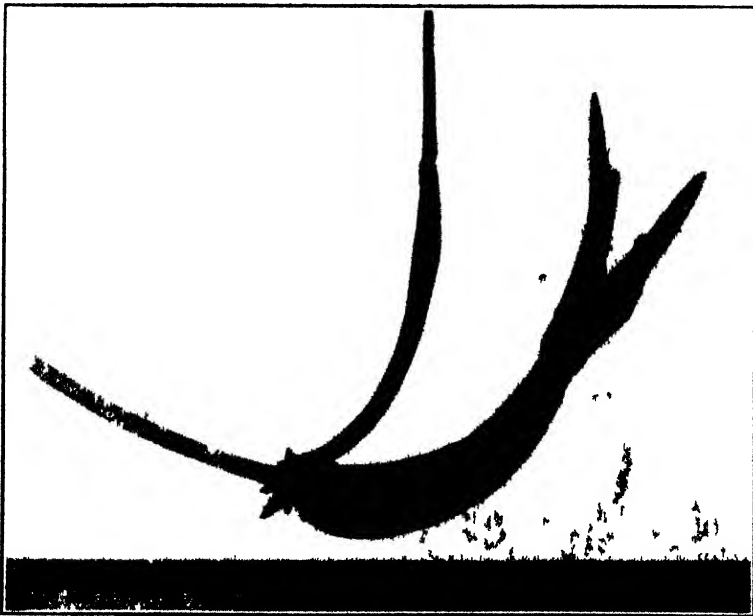
Fig 4.   Seedling tuber, two weeks after germination of the seed at 26° C   Growing point at the extreme right, the other two points being young leaves within stipular sheaths.

remains dormant for about three weeks. The first four leaves then begin to decay and if the underground parts were not examined, one would be apt to think the plants were dying. This striking behavior is a perfectly normal condition. Within three weeks after the temporary arrest in development, new tissue develops from the tuber and its enlargement is very rapid, the rhizome extending through the soil, and leaves and roots appearing at the nodes which occur at quite regular intervals. Branches of the rhizome also occur at the axils of the leaves (fig. 5).

For a preliminary study of the effect of the hydrogen ion, pH values were established at 11.5, 9.0, 6.0 and 3.3. The two extremes were replaced by plantings at 9.7 and 4.5. The plants were most easily grown at pH 9.0, the one planting being successfully carried for the seven months of the experiment. Temperatures taken at the soil surface varied from 19.5° C. in the winter months to 32° C. in the summer, the average from March 22 to July 22 being 25.4° C. The third and later leaves unrolled the laminae on the surface and during the last month of the experiment projected themselves a considerable distance above the water. The characteristic growth habit of the plant is shown in fig. 6.

Comparison of plants at pH 9.0 and pH 4.5

A comparison of the pH 9.0 plants with those grown at pH 4.5 again shows that growth at the lower pH was much more rapid. When the former were six months of age and the latter three and one-half months, the average area of laminae at pH 4.5 was 31.9 sq. cm. greater than that of laminae of pH 9.0 plants. During the following month this difference increased, the area of acid laminae exceeding that of the alkaline by 100 sq. cm. The height of the leaves above the water and the diameter of their petioles were also greater in the acid a month later. No tubers were formed at pH 4.5. Starch is ordinarily the chief food reserve in these tubers and it appears that acidity interfered with carbohydrate storage and tuber formation. The laminae, upon microscopical examination showed looser arrangement of tissue in the acid plants. This was evidently due to the rapid growth in the acid. The slower growth at pH 9.0 was at first thought to be due to a greater osmotic pressure in that solution, making absorption of materials from the water and soil more difficult, but determinations of freezing points (8) of the solutions in the pH 4.5 and pH 9 tanks showed a low osmotic pressure (fig. 7). Growth differences are thus the direct result of variations in the hydrion concentration.

The seedlings at pH 4.5 began to develop brown spots in the laminae about a month after their planting. Yellowish wilted spots appeared in the spaces between the larger veins four to five days after the laminae
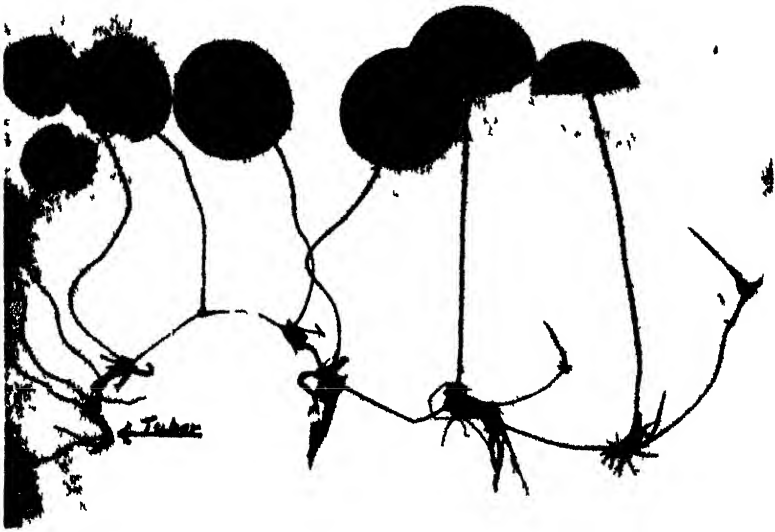
FIG 5   Seedling plant at age of seven weeks, at 26° C.   Roots from the bases of the first four leaves at the extreme left, the tuber in the second internode and the growing point of the main rhizome at the extreme right.

unrolled, then turned brown as the cells collapsed and the tissue became dry.   The dead spots spread over a larger area and soon coalesced, killing
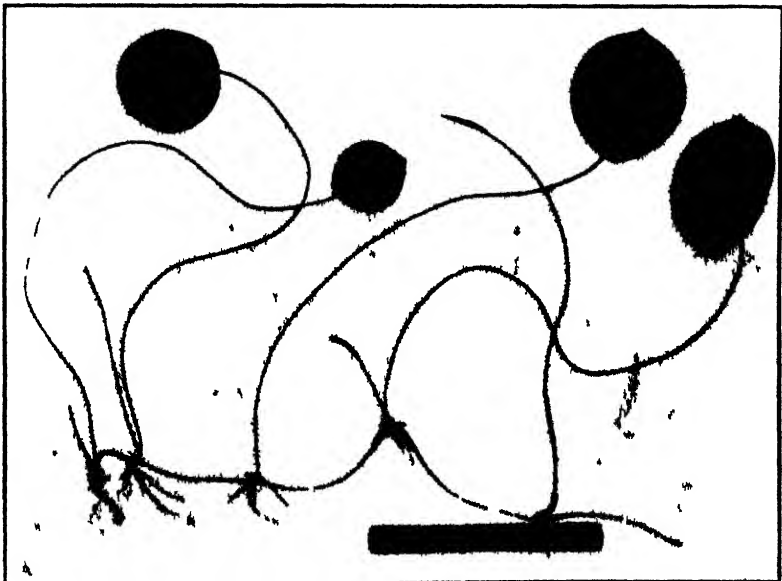
FIG. 6.   A typical plant at pH 9.0, four months from seed.
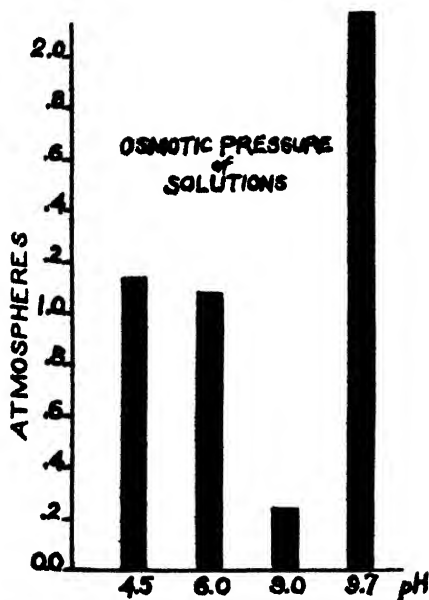
FIG. 7. Comparison of the osmotic pressures of solutions in which the plants were grown.

the laminae. Tying leaves up above the water was found to be a remedy for the trouble and from then on, nearly all were given support until the petioles of the more recent leaves raised them above the water without assistance. Sections made through spots showed no parasitic organisms. The collapse occurred first in the layer of tissue beneath the palisade. After the leaves commenced to rise above the water, unaided, there was little trouble in caring for the plants.

In the present work the plants were found to grow in a pH range from 4.5 to 9.7. Acidity, if not in excess, favors growth but the plants also develop normally at pH 9.0. Tests made on the soil and water of several natural lotus beds show a hydrion range from pH 5.2 to 8.7.

Soil type effects

The plants described above as developing at pH 7.7 were grown in a loam soil. This basin, however, contained both loam and sand, each covering one-half of the floor. The water continually running in from a tap, circulated freely to all parts of the tank. The only difference in the two portions was in the soil. This arrangement permitted a study of the influence of soil types. Equal numbers of fruits were planted in loam and in sand after having been treated with concentrated sulphuric acid. At an early date a distinct difference in the plants of the two soils became apparent. A month after planting it could very clearly be seen that there were more leaves of greater surface area on the plants in loam than those in the sand. Two weeks later this difference was even more striking (fig. 8). Tubers were found on the seedlings of both soils but were larger in the loam than in the sand. The plants remained dormant for three to four weeks and again, upon resuming growth, those in the loam greatly surpassed the others in rapidity of development.

Five months after planting there was a very decided difference, definitely showing that the soil type is probably the most important factor in the development of Nelumbo. The foregoing conclusion is based upon the

Fig. 8. Effect of sand and loam soils on growth of Nelumbo in the same pool at pH 7.7. Left, sand; right, loam. All plants six weeks old.

fact that plants in the large culture basin were bathed by the same circulating solution and hence by the same nutrient solutes. Despite this uniformity of the culture solution, the difference in the plants of the loam and sandy soils was so striking as to make inescapable the inference that the edaphic environment has a much greater effect than the aqueous environment on growth. Failure to fully appreciate this fact probably underlies many failures to establish the lotus in new localities. Whether this edaphic influence is primarily physical, nutritive or biotic remains a matter for later study. Organic soils, however, are preferable to mineral soils, especially those of siliceous type.

Conclusions

1. Despite the long delayed germination and impermeability of the fruit coats, Nelumbo plants can be grown successfully from seed.

2. The plants can tolerate a considerable hydrion range. Watergardeners and lotus enthusiasts should have little trouble as far as acidity and alkalinity are concerned in growing the plant in pools ranging from pH 4.5 to pH 9.0.

3. Difficulty in establishing *Nelumbo lutea* in new locations is probably not ascribable to low temperatures or unfavorable hydrion concentration, but rather to unfavorable edaphic factors. Mineral soils, especially those of the siliceous type, are inferior to organic types for the development of the lotus.

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LITERATURE CITED

1. BISSET, P.   The book of water gardening.   1924.
2. JONES, J. A.   Overcoming delayed germination of *Nelumbo lutea*.   Bot. Gaz. **85**: 341–343.   1928.
3. LOWRY, R. B.   The American lotus.   Flower Grower **11**: 1–5.   1924.
4. OHGA, I.   On the structure of some ancient, but still viable fruits of Indian lotus, with special reference to their prolonged dormancy.   Jap. Jour. Bot. **3**: 1–20.   1926.
5. —————.   A double maximum in the rate of absorption of water by Indian lotus seeds.   Amer. Jour. Bot. **13**: 766–772.   1926.
6. —————.   The germination of century old and recently harvested Indian lotus fruits, with special reference to the effect of oxygen supply.   Amer. Jour. Bot. **13**: 754–759.   1926.
7. —————.   A comparison of the life activity of century old and recently harvested Indian lotus fruits.   Amer. Jour. Bot. **13**: 760–765.   1926.
8. PHILIP, J. C.   Physical chemistry.   Pp. 128–130.   University Press.   Edinburgh.   1925.
9. SHAW, M. F.   A microchemical study of the fruit coat of *Nelumbo lutea*.   Amer. Jour. Bot. **16**: 259–276.   1929.
10. TRICKER, W.   Nelumbo.   Bailey's Encyclopedia of Horticulture. **4**: 2117–2118.   1917.
11. WANN, F. B., and HOPKINS, E. F.   Further studies on growth of *Chlorella* as affected by hydrogen-ion concentration.   Bot. Gaz. **83**: 194–201.   1927.
12. WHERRY, E. T.   Soil reaction in relation to horticulture.   Bull. **4**: Amer. Hort. Soc.   1926.
13. —————.   The optimum reaction ranges of crop plants.   Jour. Ind. Eng. Chem. **16**: 826–827.   1924.

TENTATIVE CLASSIFICATION OF SYMPTOMATIC TYPES OF "TOMATO POCKETS"[1]

HAMILTON P. TRAUB, W. S. HOTCHKISS,[2] AND P. R. JOHNSON

(WITH FIVE FIGURES)

It is conservatively estimated by prominent tomato growers and shippers in Texas that annually more than 15 per cent. of the Texas tomato crop is lost due to a condition commonly known as "Tomato Pops," or "Tomato Puffs." The trouble is serious in all the Southern States. At present the cause or causes of this abnormal condition are unknown, presenting a challenge to the Experiment Station worker in the South. The present paper is presented in order to bring this fruitful field for research to the attention of workers generally.
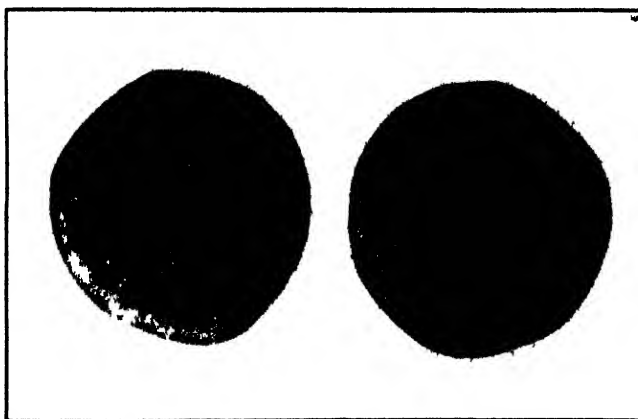
FIG. 1. Marglobe, fruit with orange-yellow exterior; symptomatic type A; marketable before edema-like symptoms develop.

Method of attack

During the season 1928, as a part of the general subject of quality in fruits, experiments were initiated at the Texas Station with the object of determining the cause or causes of this abnormal condition of tomato fruits. Although these experiments are in progress it is desirable that the problem receive attention also at other Stations since it concerns all the Southern States, California, Mexico and the Caribbean Isles. Greenhouse tomato growers in the North may also be interested. The method of attack includes a consideration of (a) the morphology and physiology of the dis-

[1] Published with the approval of the Director as Paper no. 97 of the Technical Series of the Texas Agricultural Experiment Station.

[2] Deceased, July 14, 1928.

eased fruit;[3] (b) the possible causal correlation between this abnormal condition and virus infection;[4] (c) the temperature, moisture and humidity factors, and (d) other factors, such as pollination, light, soil solution, etc., singly and in combination.

LESLEY and ROSA (2) state that one self-fertilized line was observed which produced a much larger proportion of soft and "puffy" fruits than the parent. Breeding for resistance should apparently be given consideration as a possible method of solving the problem.
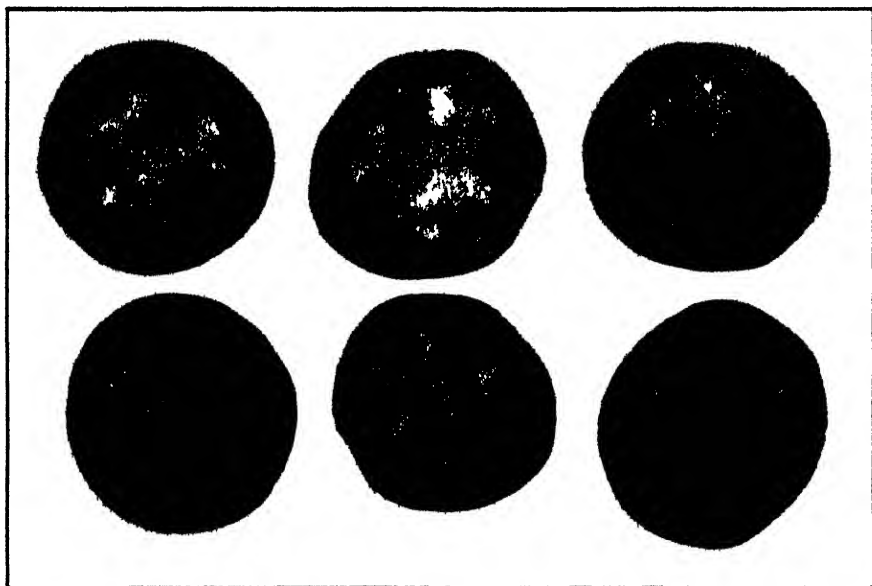
FIG. 2. Marglobe, green fruit; symptomatic type B; marketable.

Classification of types

The work as outlined is a cooperative undertaking in which many are taking part, and for this reason it was considered advisable to present a preliminary classification of the types of symptoms encountered, for the convenience of those engaged on separate phases of the research. In July, 1928, at Texas Substátion no. 2, Troup, over 5,000 diseased fruits were examined. The apparently distinct symptomatic types of the disease observed are portrayed in figs. 1 to 5, inclusive. There is apparently a gradual gradation from one type to another, but for practical reasons four types have been named.

[3] Prof. F. S. JAMISON is engaged on this problem.

[4] Drs. J. J. TAUBENHAUS, and W. N. EZEKIEL, and Mr. W. H. FRIEND are cooperating on this phase of the problem.

Aside from the possible importance of severity of attack, the symptoms apparently depend upon the stage of development of the fruit when it becomes subject to the disease:

(a). When the fruits are quite mature at the time of becoming subject to this condition they are apparently characterized by senescence of vascular bundles in the fruit, showing as white areas. The development of the ectocarp apparently is retarded in development impeding the normal

Fig. 3. Marglobe; green fruit; symptomatic type C, not marketable.

formation of lycopin. As a result, the fruit is of an orange color on the outside. At an advanced stage in the development of the fruit, the placental tissue and sometimes the meso- and endo-carpal tissues in the interior of the fruit mature almost normally, and the fruit becomes "dead ripe" when the ecto-carpal tissue is still in a yellow condition. This results in a watery interior similar to edema or dropsy. See fig. 1, symptomatic type A. This type was observed in only a limited number of cases, and is marketable before edema-like symptoms develop.

(b). Fruits affected at an earlier stage of their development apparently show large areas of opaque, degenerated tissue in the center of the fruit conspicuous as white areas radiating outward in the locular walls and placentae. See fig. 2, symptomatic type B. This type is marketable.

(c). Fruits affected at a still earlier stage of their development are apparently characterized by marked contraction of tissues, especially pla-
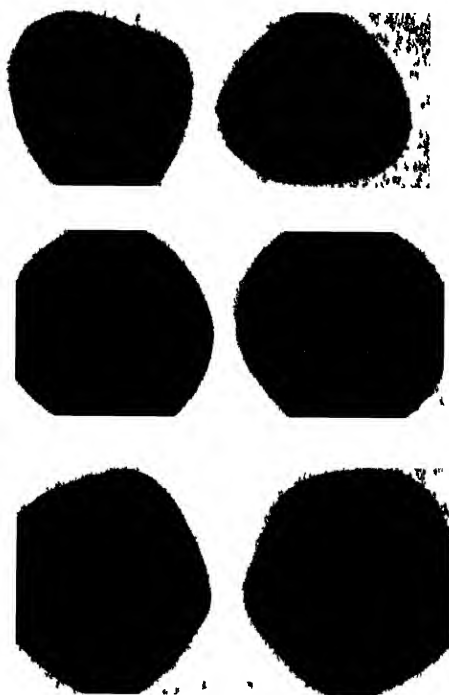
FIG. 4.   Gulf State Market; green fruit; symptomatic type C, not marketable

cental and locular-wall tissues. Both surfaces of the locular and outer pericarpal walls contract; the placental tissues contract toward the center leaving an ''open space'' or ''pocket'' and the outer pericarpal wall is tightly stretched from one locular-wall to the other. This leads to distinct angularity. The fruit is also relatively light in weight. This is the symptomatic type commonly recognized and is not marketable. See figs 3 and 4, symptomatic type C.
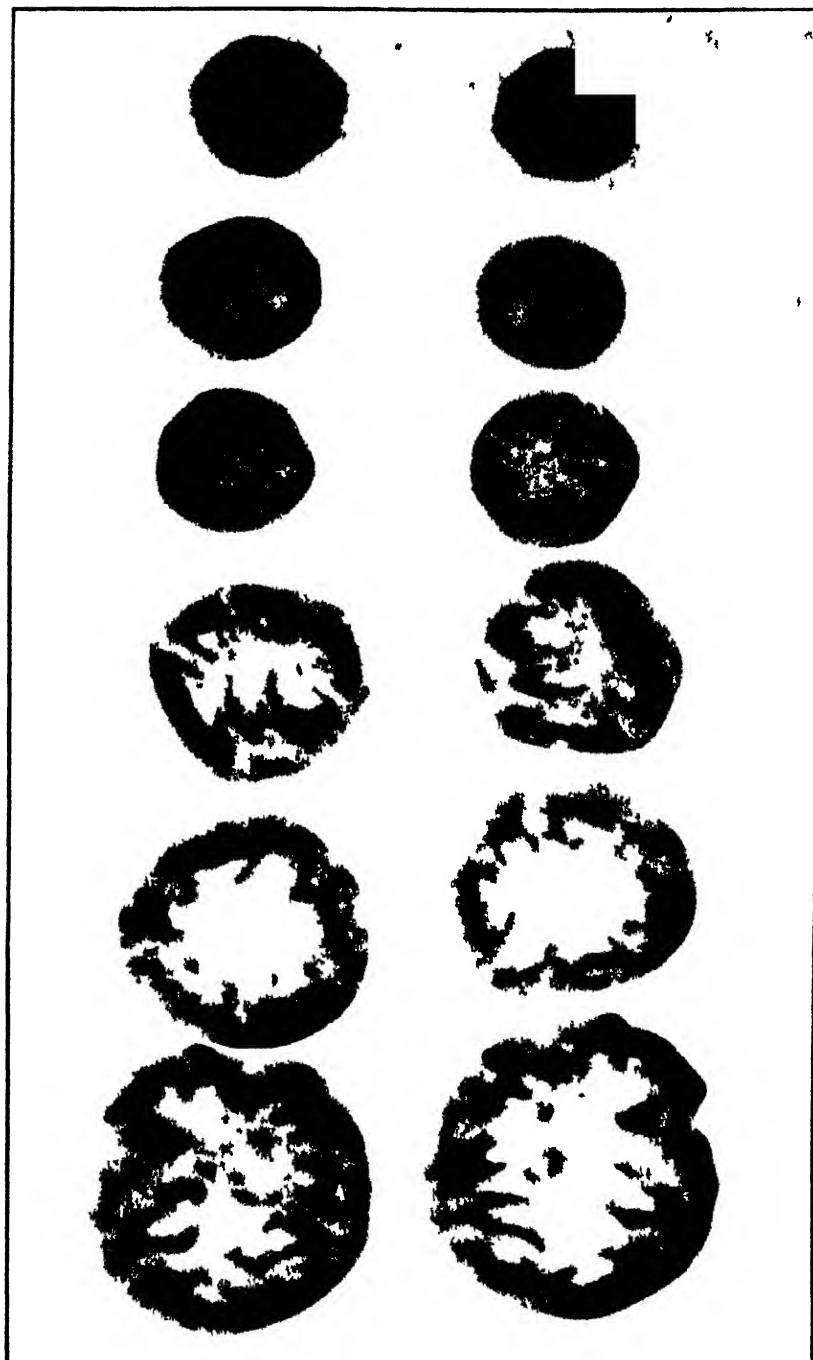
FIG 5.   Marglobe, green fruit, symptomatic type D, not marketable.

(d). Fruits affected at a very early stage are apparently characterized by total absence of seeds or reduction in number; in extreme cases, with total absence of seeds, the fruit is wholly vegetative resembling a dwarfed "witches broom." Most fruit of this type observed were small in size, but medium sized and even large specimens were observed. See fig. 5, symtomatic type D. This type is not marketable.

It should be realized, however, that this classification is merely suggestive and tentative, and that the occurrence of the last named, and possibly each of the four types may be due to different causes. Only further experimentation can settle these points.

Nomenclature

In recognition of the fact that the outstanding symptom of the typical diseased fruit, symptomatic type C, is the presence of an "open space" or "pocket" in the locules, and that there is no distention but rather a contraction of tissues, the name "Tomato Pockets" is proposed in preference to the less descriptive terms, "Puffiness" (2); "Hollow Tomato" (1); "Puffy Tomato" (4); "Puffing" (3) and "Tomato Pops" or "Tomato Puffs," (local Texas terms).

Summary

1. The subjects under investigation concerning the problem of "Tomato Pockets" are outlined.

2. A tentative classification of symptomatic types, A, B, C, and D, of the abnormal condition is presented.

3. Reasons are advanced for the acceptance of the proposed descriptive term "Tomato Pockets" in preference to less descriptive local terms.

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LITERATURE CITED

1. FRIEND, W. H. (Private communication, July 30, 1928, on observation of "Hollow Tomato" at Texas Substation no. 2, Troup, in 1921.)
2. LESLEY, J. W., and ROSA, J. T. Improvement of tomatoes by selection. Hilgardia 2:·25–45. 1926.
3. TAUBENHAUS, J. J. Tomato diseases. Texas Agr. Exp. Sta. Ann. Rept. 1926. p. 47. 1927.
4. WEBER, G. F., and RAMSEY, G. B. Puffy tomato. Tomato diseases. Florida Agr. Exp. Sta. Bull. 185. 1926.

THE RÔLE OF MANGANESE IN THE NUTRITION OF LEMNA[1]

NORMAN ASHWELL CLARK AND CLAUDE L. FLY

(WITH THREE FIGURES)

The place of manganese in the nutrition of the green plant has been investigated in detail for some years, and numerous claims have been advanced with regard to its function, but there does not seem to be any considerable consensus of opinion as to its effect on the composition of the tissues or on the reproduction of the plant. There seems to be an equal uncertainty whether manganese should be classed as one of those elements which have been claimed as essential for growth (although in small quantities), or if it should be put with those which stimulate but are not absolutely necessary for the existence of the plant.

In 1920 OLARU published a thesis on the place of manganese in agriculture (15) and reviewed the work done throughout the world up to that time. In almost all cases manganese had proved favorable to the development of plants in soils, although large quantities were decidedly toxic. In liquid cultures the addition of suitable quantities of manganese stimulated both germination and development.

OLARU showed that this energetic action of manganese had been explained by the many different investigators, in almost as many different ways, including among others: catalysis of chemical action in the cell (BERTRAND); oxidation of toxic substances in the leaves (LOEW); production of chlorophyll (VAN DORN); rôle in photosynthesis (STOKLASA); stimulation of assimilation of elements (ROUSSET); chemical action on soil salts (MENOZZI); increased solubility of calcium salts and silicates (BERNARDINI); increased oxidation (SCHREINER); oxidation and stimulation of bacteria (SKINNER); permeability of protoplasm of cell to salts of calcium and magnesium (KELLY); ratio of iron to manganese (PUGLIESE).

By 1925 the amount of information had increased but without altering to any large extent the conclusions of OLARU. Influenced by the discussion on the essential nature of the vitamins in animal life, investigators gave more attention to the place of manganese as a possible essential element in plant life, in contrast to its stimulating action. BRENCHLEY (5) in her monograph on Inorganic Plant Poisons and Stimulants in 1927 sums up that manganese has a stimulating action on growth in small quantities, but large amounts are toxic to plants. The precise way in which this stimulation is accomplished is not known, and the "essential" nature of the element is as yet uncertain.

[1] Contribution from the Department of Chemistry, Iowa State College.

Several workers have recently reported experiments on plants for which manganese seemed an essential. HAAS and REED (12) found that with young orange trees they could not get good growth in culture solutions commonly considered "complete," when the young trees were grown for long periods in these solutions. Manganese was among the elements which it was necessary to add in traces to remove this condition. BISHOP (4) in Australia noted the presence of manganese in 25 species of Eucalypts. In these and in wheat, barley, maize, vegetables and fruits, the manganese was concentrated in the parts of the plants where there was the greatest chemical change. In his sand cultures he found approximately 5 parts per million was the optimum concentration in the nutrient solution supplied, but that neutralizing the acid solution with $CaCO_3$ enabled plants to grow in 50 ppm.

McHARGUE (14) in 1927 reported the occurrence of a number of elements, including manganese, in Kentucky blue grass. From earlier experiments he had concluded that manganese is essential for the growth of plants, and is connected with chlorophyll or protein formation, or possibly with the stimulation of enzymes which split fats, sugars and starches, and render them available for young seedlings. He suggests here that it is the manganese which causes the deep blue color of the blue grass, and he proposes a theory connecting that element with vitamin production in plants. As most of the copper, manganese and zinc occurs in the germ of the seed, it may be connected with those vitamins which are found in the germ of the seed. Similarly the mineral elements supply the vitamin-forming material necessary for the growth of plants, and may become upon resynthesis the vitamin factors necessary for animal growth.

A number of these points can be studied very well in nutrient solutions. Aso (3) in 1902 used water cultures and stated that plants could develop normally in the absence of any trace of manganese, while WEIS (17) more recently states that manganese could not prevent chlorosis and that it did not influence the plants in solution. These conclusions are not in accord with those of HAAS, BISHOP or McHARGUE.

The value of water plants for nutrient studies has been generally recognized, but the fact that they are specially adapted to the investigation of manganese has not been made use of to any extent. GÖSSL (11) showed that marsh and water plants generally gather up considerably more manganese than land plants; *Lemna trisulca*, for example, contained large amounts. In the experiments reported below, *Lemna major* (*Spirodela polyrhiza*) was used. As collected from ponds in central Iowa it showed a considerable amount of manganese in its composition.

Conditions under which the *Lemna* plants grow have been investigated in this laboratory for several years. In 1924 CLARK and ROLLER (10)

showed that the plants, if given a suitable medium of inorganic constituents, did not need the addition of organic matter in order to reproduce and to keep healthy, and that the "auximones" suggested by BOTTOMLEY were possibly of the nature of stimulants, but could not be classed as essentials for the growth of plants. Effects of intensity and duration of light on the reproduction were reported by CLARK in 1925 (6) and the influence of hydrogen ion concentration was investigated in 1926 (7). The medium developed in this way has been used by ASHBY and his coworkers (1, 2) in London with considerable success in the growth and reproduction of *Lemna minor*. A general review of some of the problems connected with vitamins and plant growth was given by CLARK in 1929 (9).
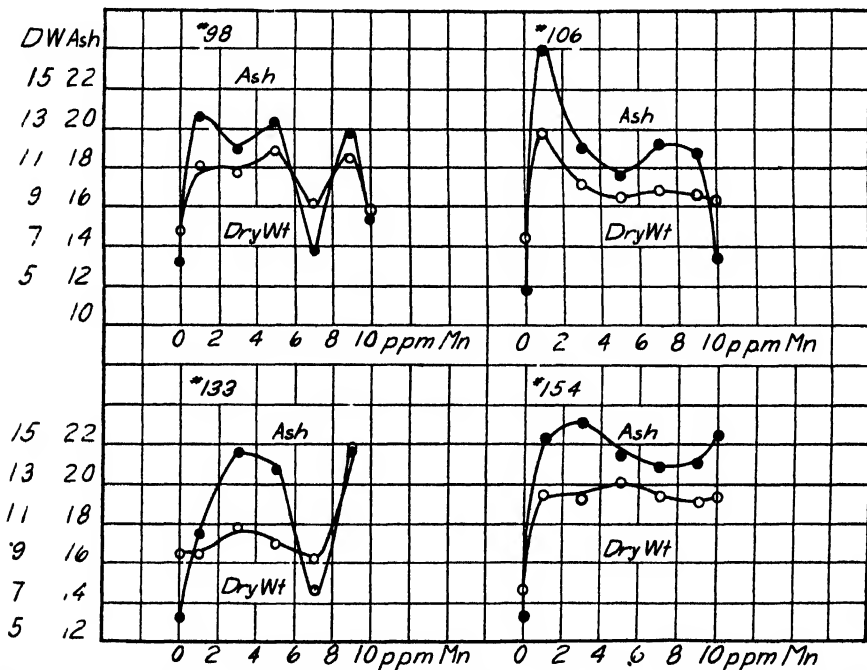
FIG. 1. Average dry weight and ash in plants with concentration of manganese 0 to 10 ppm. in the solution. Scale for ash weight is 10 × dry weight.

The nutrient solution was used as indicated by CLARK (6) and the initial pH was adjusted when necessary to 4.8. Salts were purified by recrystallization as described by CLARK (8). The technique followed was altered slightly by substituting Erlenmeyer flasks for beakers, and these were closed with loose plugs of cotton wool. The flasks were placed in a thermostat in which the temperature was kept at 25° C. and exposed to sunshine; no artificial light was used. Controls were kept at all times in the standard solution along with the other flasks, and conclusions drawn from comparison

with these controls. As the light varied at different periods of the year, cultures grown at different times were compared only through the controls.

The stock plants were grown in the inorganic solution free of manganese for several months before being used for the experiments. They had thus passed through some 40 or 50 generations without access to manganese. The number of fronds was counted and the reproduction constant, K, calculated from $\log_{10}N - \log_{10}N_0 = K(t - t_0)$ (6). The fronds were dried and the average weight found. The ash and volatile matter were also determined. The method for obtaining the dry weight is somewhat different from that outlined by SU (16), who placed the plants for 30 minutes in a vacuum of 5 mm. at 50° C. In this laboratory the plants are dried in micro-cru-
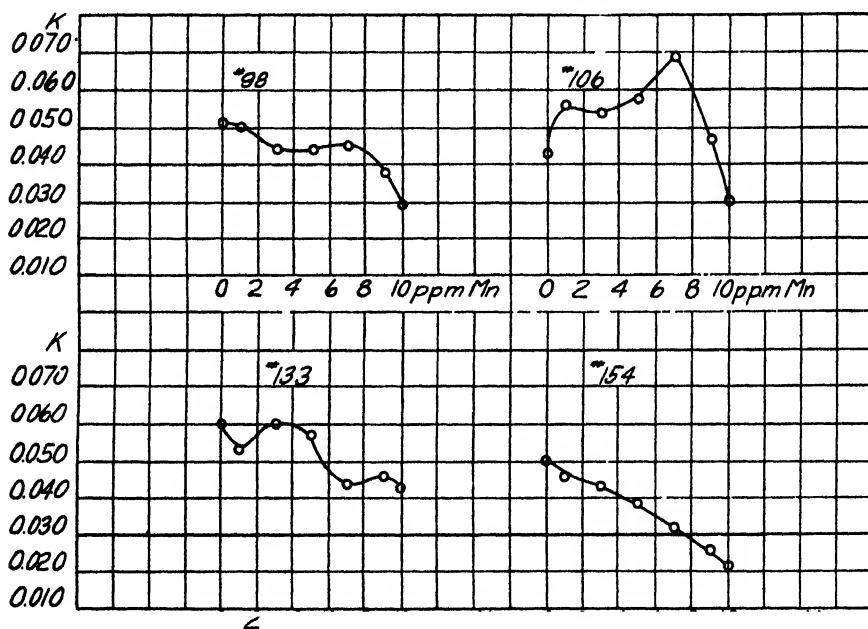
FIG. 2. Reproduction constant, K, for concentration of manganese from 0 to 10 ppm. in the solution.

cibles of platinum under a partial vacuum in a stream of dry air at 45° C. The platinum crucibles were placed in porcelain crucibles in the desiccating chamber, and when the plants were dry (half an hour or over night showed no change in weight) the lids were placed on the porcelain crucibles, and the whole transferred to a sulphuric acid desiccator until room temperature was reached; or the desiccating chamber was allowed to cool and the platinum crucibles transferred to a closed weighing bottle of known weight which was kept in the case of the Ainsworth microbalance (13). It was found necessary to have the weighing bottle in the balance. (If kept in the

drying chamber the condensation of moisture during the weighing is very variable.)

Figure 1 shows some typical curves representing the average dry weight and ash content of the fronds both in the medium without manganese and with additions of manganese chloride to make concentrations of that element from 1 to 10 parts per million. It will be seen that there is in every case a rapid rise in both dry weight and ash as the manganese is added until a maximum is reached at about 1 or 2 ppm., after which both are irregular to 10 ppm. Above 10 the manganese concentration becomes increasingly toxic and the plants die off rapidly in the higher concentrations. The rate
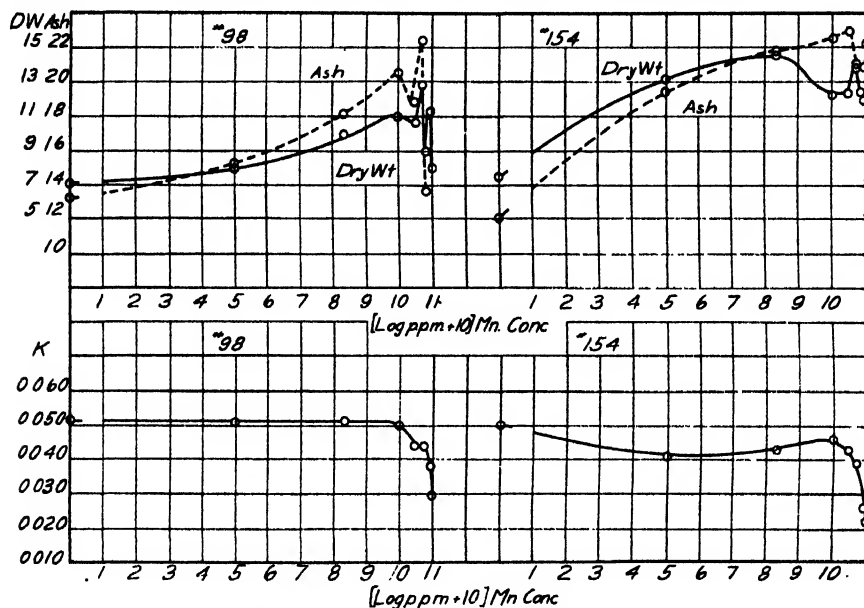
FIG. 3. Average weight and ash, and reproduction constant, K, with logarithm (plus 10) of the ppm. of manganese in the solution, as abscissa. Scale for ash weight is 10 × dry weight.

of reproduction, however, as represented by the constant, K, does not show any regular increase with the addition of manganese. This is shown in figure 2. From 1 to 10 parts per million there is a tendency for K to decrease, and in the region of these concentrations the plants frequently show loss of roots and brown spotting.

That about 1 ppm. of manganese is the concentration for maximum dry weight and maximum ash content under these conditions, is clearly shown in figure 3, where the abscissa is the logarithm of the ppm. When the average of eight or ten sets of weighings was used, the maximum for both dry weight and ash was very close to 1 ppm. No such rise is shown in the repro-

duction constants in figure 3 and the average of eight or ten sets gave an almost horizontal line until close to 1 ppm. The influence of the manganese is therefore probably upon the assimilation of the plant and not upon the reproductive function, at least until the region of toxic concentration is reached.

It was possible to obtain more information concerning the effect on composition, by determining the approximate green weight of the plant. This weight was obtained by washing the fronds through two changes of distilled water, and removing the surface water by pressing lightly between filter papers. The plants were then placed in the platinum crucibles and these weighed inside the closed weighing bottles, as described for the dry weight determination. The green weight showed a close parallel to the dry weight. The difference between the green and dry weights gives a fairly good approximation to the water content, and the volatile matter on ignition is easily obtained. When these were graphed on the logarithm of the concentration of manganese it was seen that the ratio of water, volatile matter and ash varied very little up to 1 ppm. of manganese; on the average there was an indication of a slight tendency for the ash to increase and the water to decrease. From 1 ppm. up, there was the same irregularity with increasing concentration of manganese as shown in the dry weight and ash.

The lower limit of the toxic concentration of manganese under these conditions can be assumed to be somewhere near 1 ppm. Below this concentration the addition of the element to the inorganic salt solution increased both weight and size of plant. It did not, however, have any effect upon the rate of reproduction of the Lemna, neither was there any marked alteration in the ratio of water in the plant to volatile matter or ash. Above 1 ppm. of manganese, reproduction, dry weight, ash weight and composition were all irregular and toxicity increased rapidly with higher concentration.

With regard to the essential nature of manganese we can find no trace of that element in the ash of the plants grown in the inorganic medium unless manganese is added to the solution. The water, after the third distillation (in pyrex glass) is kept in pyrex containers and the plants are grown in pyrex flasks. Boron and silicon may be obtained from this glassware by the plant, and there is a chance that some manganese could be obtained in this way also, although no trace of manganese could be found by evaporating 20 liters of the water which had stood in the glass containers for several weeks; also the ash of the plants grown in the stock solution gave no signs of its presence.

There is some indication that the plant will adapt itself gradually to a concentration of manganese which proves injurious at first; but whether the effect of the manganese, or the element itself, can be carried over for several

generations after the plant goes back to a lower concentration or a manganese free solution, is as yet doubtful, although some results point to that conclusion.

Summary

1. *Lemna major* has been grown in a medium of inorganic salts with and without the addition of manganese.

2. There is no indication that manganese is an essential element in the nutrition of the plant.

3. The size and weight of the fronds increase up to about 1 ppm. of manganese, but the ratio of ash, volatile matter and water is little affected. There is no increase in the rate of reproduction.

4. Above 1 ppm. the concentration becomes gradually more toxic, and reproduction, size, weight and ash all become very irregular.

5. Indications point to the probability of the adaptation of the plant to manganese.

LITERATURE CITED

1. ASHBY, E.   The interaction of factors in the growth of *Lemna*. 3. The interrelationship of duration and intensity of light. Ann. Bot. **43**: 333–354. 1929.

2. ——————, BOLAS, B. D., and HENDERSON, F. Y.   The interaction of factors in the growth of *Lemna*. 1. Methods and technique. Ann. Bot. **42**: 771–782. 1928.

3. ASO, K.   On the physiological influence of manganese compounds on plants. Bull. Coll. Agr. Tokyo Imp. Univ. **5**: 177–185. 1902.

4. BISHOP, W. B. S.   The distribution of manganese and its importance in plant metabolism. Australian Jour. Exp. Biol. Med. Sci. **5**: 125–141. 1928.

5. BRENCHLEY, W. E.   Inorganic plant poisons and stimulants. 2nd ed. Cambridge, 1927.

6. CLARK, N. A.   The rate of reproduction of *Lemna major* as a function of intensity and duration of light. Jour. Phys. Chem. **29**: 935–941. 1925.

7. ——————.   Plant growth-promoting substances, hydrogen-ion concentration and the reproduction of *Lemna*. Plant Physiol. **1**: 273–279. 1926.

8. ——————.   Purification of chemicals for use in the preparation of nutrient solutions. 1. The crystallization of phosphoric acid. Proc. 1st Intl. Congress Soil Sci. **3**: 300–304. 1927.

9. ——————.   Vitamins and the world of plants. Sci. Monthly **28**: 343–348. 1929.

10. ——————, and ROLLER, E. M. "Auximones" and the growth of the green plant. Soil Sci. **17**: 193–198. 1924.

11. GÖSSL, J. Über das Vorkommen des Mangans in der Pflanze und über seinen Einfluss auf Schimmelpilze. Beih. Bot. Cent. **18**: 119–132. 1905.

12. HAAS, A. R. C., and REED, H. S. Significance of traces of elements not ordinarily added to culture solutions, for growth of young orange trees. Bot. Gaz. **83**: 77–84. 1927.

13. HIXON, R. M. Microbalance made in U. S. A. News ed. Jour. Ind. Eng. Chem. **3**: 5. 1925.

14. McHARGUE, J. S. Significance of the occurrence of manganese, copper, zinc, nickel and cobalt in Kentucky blue grass. Jour. Ind. Eng. .Chem. **19**: 274–276. 1927.

15. OLARU, D. A. Contribution à l'étude du rôle du manganèse en agriculture. Son influence sur quelques microbes du sol. Université de Paris. 1920.

16. SU, M. T., and ASHBY, E. The interaction of factors in the growth of *Lemna*. 2. Technique for the estimation of dry weight. Ann. Bot. **43**: 329–332. 1929.

17. WEIS, F. Cultures in different nutrient solutions with special reference to the influence of manganese and of the hydrogen-ion concentration. Translated title. Aarsskr. kgl. Veter. Landbohöjsk. 239–280. 1919. Chem. Abs. **14**: 1841. 1920.

A MICROELECTRODE FOR THE RAPID DETERMINATION OF THE HYDRION CONCENTRATION OF EXPRESSED JUICES FROM SMALL AMOUNTS OF PLANT TISSUE[1]

B. C. BRUNSTETTER AND C. A. MAGOON

(WITH ONE FIGURE)

Introduction

In 1928, while working with Doctor J. S. CALDWELL, of the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. D. A., it became necessary to obtain accurate data on the hydrion concentration of the outer and inner wall, septa, placentae, and pulp surrounding the seeds, of individual tomato fruits at various stages of development. For very young fruits (less than 15 days after fertilization) the material available was obviously limited and called for some type of microelectrode. Since numerous determinations were required, and each determination had to be performed quickly to prevent or minimize possible changes in the acidity of the expressed juice of the fruit part, a rapid as well as accurate technique was essential.

To meet these needs, the excellent microelectrode devised by BODINE and FINK (5) was chosen. Their apparatus consists essentially of a small electrode vessel, terminating in a capillary tube, and mounted on a modified microscope stand in such a way that the electrode vessel may be lowered by the coarse adjustment of the microscope until the capillary tube, filled with the liquid to be examined, makes contact with a saturated KCl solution contained in a U-tube connected with a calomel electrode. A platinum electrode is mounted at the top of the stand in such a way that it may be readily lowered or raised in the gas-tight electrode vessel. A side-arm on the electrode vessel provides for the entrance of hydrogen.

The amount of liquid required for a determination depends on the degree of capillarity of the end of the electrode vessel. In the form used by BODINE and FINK, only 0.015–0.020 cc. is required: they found it possible to make five to six acidity determinations on the blood of a single grasshopper without killing the animal. The samples could be drawn into the electrode vessel without significant exposure to air.

Later BODINE (4) used a form of this electrode in which the capillary tube of the vessel was finer, reducing the capacity to 0.01 cc.; with it he

made determinations of the acidity of the contents of individual fertilized or unfertilized ova of the marine fish, *Fundulus heteroclitus.*

Description of modified electrode

Two changes have been made in this microelectrode which increase its efficiency, as far as its use with the expressed juices of plant parts is concerned. For filling the electrode vessel, BODINE and FINK relied on capillarity plus the effect of shutting off the flow of hydrogen, when the tip of the capillary met the liquid under investigation. This method is suitable when the amount of juice available is less than 0.1 cc. and when the juice is free of cellular débris. In expressed plant juices, however, there is usually more or less débris present which tends to clog the entrance of a fine capillary tube. Since a comparatively large diameter reduces the capillarity of the tip, another means of filling the end of the electrode vessel is necessary. Consequently, a side tube, equipped with a stopcock and a small rubber bulb, was fused on the electrode vessel opposite the hydrogen inlet. Compression of the bulb expels some of the hydrogen in the electrode vessel so that as the pressure on the bulb is released, liquid is drawn up into the capillary tube when its tip is dipped under the liquid being investigated. By this means the amount of juice drawn up into the vessel can be closely regulated. Moreover, washing the electrode vessel between determinations is very much facilitated.

In place of the vaseline seal used by BODINE and FINK, a mercury seal has been substituted. The sudden changes of pressure involved in filling and emptying the tip of the electrode vessel with plant juices, many of which are quite viscous, have made this modification necessary. The mercury seal holds in cases where a gas passage would be forced through a vaseline seal.

Figure 1 is a line drawing, one-half actual size, of the electrode mounted on a microscope stand modified by removing the substage condenser and cutting away the central portion of the tube. Electrode A is a mercury-filled glass tube in the end of which a straight platinum wire J is fused; it is centered by means of de Khotinsky cement in a small brass collar C, machined to screw into the milled brass top D which fits tightly into the upper end of the sawed-off microscope tube F. A one-hole rubber stopper G holds the upper end of electrode vessel Q in place. As the drawing shows, the tube A ends in a mercury seal MS. The side-arm O for filling the electrode vessel is equipped with a stopcock and a small rubber bulb P. The opposite side-arm N is the hydrogen inlet with a stopcock handily placed for controlling the gas flow. The central portion of the electrode vessel I is continuous with the upper tube Q, which has a small hole R, for the pur-
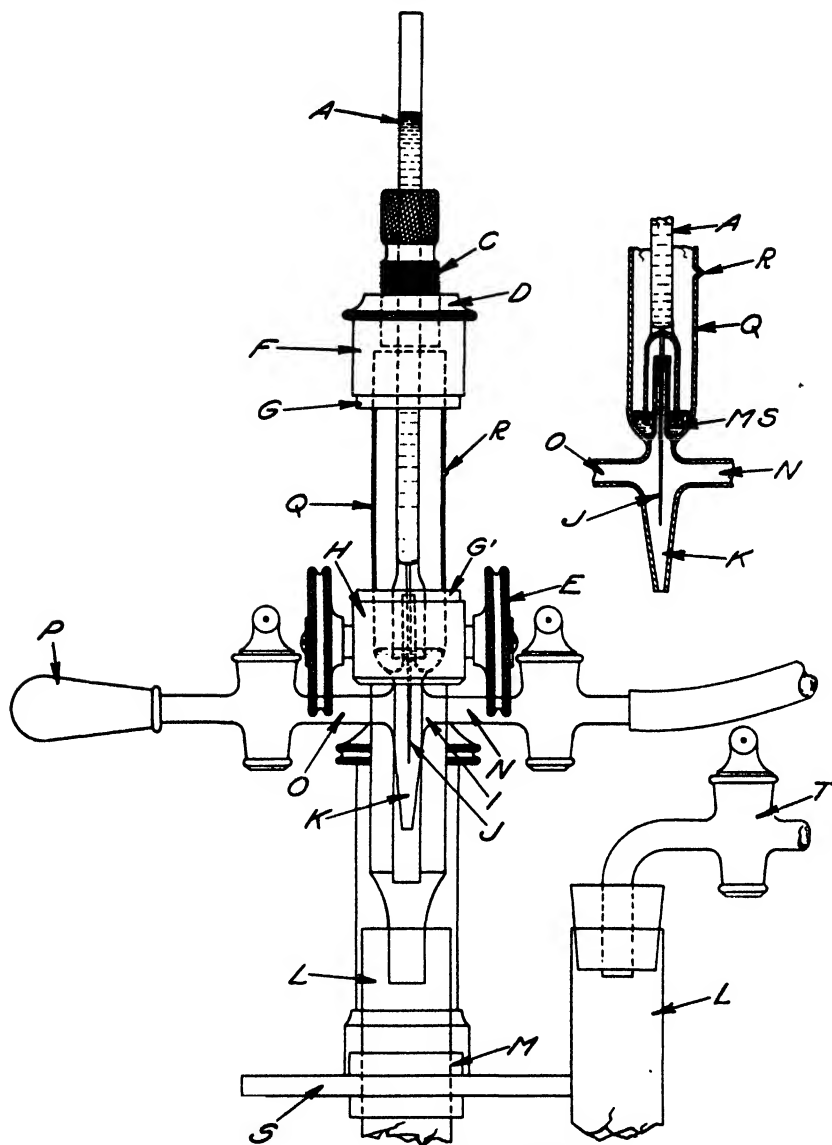
FIG. 1.   The modified microelectrode.

pose of equalizing the pressure inside and out.   The lower portion of the electrode vessel is held by means of the rubber stopper G' in the lower end of the microscope tube H.   By means of the coarse adjustment E the open capillary tip K can be raised from or lowered into the U-tube L which contains saturated KCl solution and connects with the colomel electrode T partly shown.   The U-tube is held in the stage S of the microscope by means of the rubber stopper M.

Technique

The technique employed is as follows: The plant part to be analyzed is separated from the surrounding tissue and the juice is then expressed through cheesecloth into a small porcelain crucible. This is placed on top of one end of the U-tube and the electrode vessel which has been previously flushed out with hydrogen is lowered to make contact with the expressed juice. At the moment of contact the hydrogen is shut off and some hydrogen is expelled by compressing the bulb P. The pressure on P is then slowly released; when the rising liquid has reached the desired height, its ascent is checked by closing the stopcock on the arm O. This rinsing liquid is then expelled by hydrogen when the stopcock on the side-arm N is opened. After rerinsing, a sample is drawn up for analysis. The crucible is then removed and the electrode vessel is lowered by means of the coarse adjustment E to make contact with the saturated KCl solution. By means of the screw C the tip of the electrode is adjusted so that it just makes contact with the surface of the liquid. The potential is then read as quickly as possible by means of a suitable potentiometer.[1] Equilibrium is obtained in less than a half minute. (That equilibria reached so quickly are not "pseudo-equilibria" is shown (1) by the fact that a reading taken two or three minutes later, with the electrode just touching the surface of the liquid, agrees within two millivolts with the initial reading, and (2) by the excellent agreement of the initial reading of the microelectrode with a check reading by the Bailey electrode.) The electrode vessel is then lifted out of the saturated KCl solution and its contents are expelled into a small crucible by flushing the vessel out with hydrogen.

Washing the electrode vessel is performed in the same manner, and two rinsings with distilled water and one with the liquid to be analyzed may be quickly made. One determination followed by three rinsings requires on the average four minutes.

In working with plant juices it has been thought advisable for the sake of safety to replatinize frequently. The technique is quite simple. By unscrewing the collar C with the attached electrode A, the electrode may be lifted out entirely from the electrode vessel and cleaned by heating in a flame. (BILLMANN (2) advises against the use of a gas flame, and recommends the flame of either a benzene blow-torch or alcohol vapor lamp.) It is then returned to position and platinic chloride solution is drawn up into the electrode vessel, an accessory electrode is inserted into the platinic chloride solution in the crucible and replatinization is accomplished in the manner recommended by CLARK (6); a thin coating only is preferable. As a check on the nature of the coating, the electrode is tested with M/20

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[1] In the work here described, a Leeds and Northrup Type K potentiometer was used.

potassium acid phthalate solution. As DRAVES and TARTAR (7) have shown, accurate readings may be obtained on this solution with the hydrogen electrode if its coating of platinum black is thin. A thick coating may result in upward drift of potential due, presumably, to reduction of the potassium acid phthalate.[2] After replatinization the vessel is thoroughly washed by rinsing at least four times. The whole process can be performed in less than five minutes.

Replatinization *in situ* is necessary in order to avoid the danger of scraping some platinum black off the electrode as it is inserted into the electrode vessel. If the end of the electrode vessel is drawn out into a fine capillary, the resistance of the platinic chloride solution becomes correspondingly great, so that a battery of sufficiently high voltage must be employed in order to replatinize readily.

This microelectrode can be used not only as a hydrogen, but as a quinhydrone electrode. The platinum electrode is cleaned by flaming it and then returned to position. Some quinhydrone is mixed with the expressed plant juice, the mixture is then drawn up into the electrode vessel so that the platinum electrode is deeply immersed. After making contact with the saturated KCl solution, the voltage is measured as usual. In this way the same piece of apparatus may be used to obtain duplicate readings by two different methods. It should be mentioned in passing, however, that the quinhydrone electrode is inapplicable to many plant juices, a rapid drift of potential occurring so that the pH calculated from a reading taken thirty seconds after mixing quinhydrone and juice differs materially from the pH obtained by the hydrogen electrode.

Discussion

In table I a few illustrations are given of some of the uses made of the modified microelectrode in this laboratory.

The acidities are given not only in terms of pH but also, to facilitate comparison and to obtain a mean with its probable error, as acidity units per liter, following the method proposed by WHERRY (9, 10, 11). One acidity unit is 1×10^{-7} gm. hydrogen ion per liter. To convert acidity units into hydrion concentration expressed as a normality, it is only necessary to point off the acidity units seven places to the left of the decimal place.

Determinations on the grapes, using the apparatus as a quinhydrone, then as a hydrogen, electrode, checked closely. The first determination on

[2] However, BLACKADDER (3) has obtained correct values (pH 3.97) on M/20 potassium acid phthalate solution using very heavily coated platinum electrodes. It seems that a thinly coated electrode invariably gives correct results tested on this standard but a heavily coated electrode may or may not do so.

TABLE I

ACIDITY VALUES, OBTAINED BY THE MODIFIED MICROELECTRODE, OF SOME EXPRESSED
PLANT JUICES

MATERIAL	ACIDITY	
	pH	Acidity units[1] per liter
Grapes (Thompson's Seedless		
Mean acidity of series of 25 grapes, full ripe	3.84	1449 ± 41[2]
Acidity of juice from composite sample of 50 grapes ..... (1)	3.83	1479
(2)	3.85	1413
Strawberries		
A. Diameter of berry = 13.0 × 11.0 mm.		
Taken from a sample at a stage following petal fall......	3.42	3802
B. Diameter of berry = 15.0 × 14.0 mm.		
Taken from a sample in which the berries were mak-		
ing a rapid volume increase...........................	3.03	9333
Tomatoes (Marglobe)		
A. Picked 12 days after fertilization.		
Weight = 11.61 g.		
Diameter = 29.6 × 24.8 mm.		
Wall	5.26	55
Septa and placentae	5.13	74
Pulp	4.79	162
B. Picked 28 days after fertilization.		
Weight = 28.58 g.		
Diameter = 41.3 × 34.4 mm.		
Wall	4.95	112
Septa	4.96	110
Placentae	4.80	159
Pulp	4.63	235

[1] cH is expressed, as recommended by WHERRY (9, 10, 11) as acidity units per liter, the unit being 10^{-7} gm. per L.

[2] Coefficient of variation: 21.34 per cent.

the composite sample of grapes was made by the former, the second by the latter, electrode. The figures on individual grapes were made by the quinhydrone electrode, but in all other analyses, the hydrogen electrode was used.

It is obvious that analyses of the acidities of an adequate number of individual fruits, instead of a composite sample of fruits has the advantage that not only the mean, but the distribution about the mean is obtained. Data of this kind are essential to throw light on the important question as

to what extent the acidity of a plant part at a given stage of development can be considered as a physiological constant.

The strawberries were a composite sample of several varieties of the everbearing type. This sample was picked and graded roughly into various stages of development by G. F. WALDO, of this Office. The data on strawberries and tomatoes, which are amply confirmed by other work not reported here, illustrate the marked increase in acidity which occurs in the early stages of growth of fruits.

The modified microelectrode has also been of considerable service in this laboratory in quickly checking the BAILEY (1) and the HILDEBRAND (8) types of electrodes. In this connection, it has been tried with satisfactory results on apples, peaches, tomatoes, and the leaves and the petioles of the sugar beet, the macro- and the microelectrodes generally agreeing within 0.05 pH.

To illustrate the value of the modified microelectrode in routine acidity analysis, where rapidity as well as an accuracy greater than that of the colorimetric method is desired, it may be mentioned that in one working day, 109 determinations of hydrion concentration of the juices of the wall and pulp of 51 tomatoes were made by the modified microelectrode, the samples being prepared by two assistants.

Summary

The microelectrode of BODINE and FINK is modified in two important respects to make it more suitable for dealing with plant juices: (1) a mercury seal is substituted for a vaseline seal and (2) filling and washing of the electrode vessel is secured by fusing a side-tube, equipped with stopcock and rubber bulb, opposite the hydrogen inlet. The electrode can function either as a hydrogen or as a quinhydrone electrode. The amount of liquid required for measurement depends on the size and shape of the electrode vessel and may vary from 0.25 to 0.01 cc. Data illustrative of the use of the microelectrode in plant physiological research are given.

U. S. DEPARTMENT OF AGRICULTURE,
    WASHINGTON, D. C.

LITERATURE CITED

1. BAILEY, C. H.   A simple hydrogen electrode.   Jour. Amer. Chem. Soc. **42**: 45–48.   1920.

2. BIILMANN, E.   On the measurements of hydrogen-ion concentrations in soil by means of the quinhydrone electrode.   Jour. Agr. Sci. **14**: 232–239.   1924.

3. BLACKADDER, T.   Ripening of hydrogen electrodes.   Jour. Amer. Leather Chem. Assoc. **20**: 17–19.   1925.

4. BODINE, J. H. Potentiometric studies on intracellular pH values of single *Fundulus* egg cells. Jour. Gen. Physiol. **10**: 533–540. 1927.

5. ————, and FINK, D. I. A simple micro-vessel with electrode for determining the hydrogen-ion concentration of small amounts of fluid. Jour. Gen. Physiol. **7**: 735–740. 1925.

6. CLARK, W. M. Determination of hydrogen ions. Williams and Wilkins, Third ed. 1928.

7. DRAVES, C. Z., and TARTAR, H. V. Instability of phthalate solutions toward the hydrogen electrode. Jour. Amer. Chem. Soc. **47**: 1226–1230. 1925.

8. HILDEBRAND, J. H. Some applications of the hydrogen electrode in analysis, research and teaching. Jour. Amer. Chem. Soc. **35**: 847–871. 1913.

9. WHERRY, E. T. The statement of acidity and alkalinity, with special reference to soils. Jour. Wash. Acad. Sci. **9**: 305–309. 1919.

10. ————. New method of stating hydrion concentrations. Bull. Wagner Free Inst. Sci. **2**: 59–64. 1927.

11. ————, and ADAMS, E. Q. Methods of stating acidity. Jour. Wash. Acad. Sci. **11**: 197–199. 1921.

METHODS FOR THE QUANTITATIVE EXTRACTION AND SEPARATION OF THE PLASTID PIGMENTS OF TOBACCO

PAUL D. PETERSON[1]

Introduction

During the past year, in making pigment studies of several types of mosaic on tobacco, the writer has used rather extensively the methods of WILLSTÄTTER and STOLL as modified by SCHERTZ (1) for the extraction and separation of chlorophyll, carotin, and xanthophyll. The method has proved to be effective and sufficiently accurate for comparative studies of the mosaic diseases of tobacco. It has been possible, however, to make several short cuts in the method without the sacrifice of accuracy, and to eliminate, at the same time, the hazard of emulsion formation in the separation of carotin and xanthophyll. By making a slight change in the method it has been possible also to obtain and measure that portion of the green pigments which is in an altered condition in the plant tissues at the time of extraction. This fraction either has been lost in other methods of extraction and separation (1) or has been measured as part of the unaltered chlorophyll (3).

Materials and methods

The method is standardized on the basis of ten-gram samples of fresh leaf tissues, stems and petioles being excluded from the samples. With large leaves, such as those of tobacco, it is desirable also to exclude midribs and the large lateral veins. The leaves are thoroughly ground with quartz sand in a mortar and transferred with acetone to a Büchner funnel for subsequent extraction.

The pre-wash of the finely ground leaf tissues with 30 per cent. acetone is omitted. Most of the substances removed from the leaf solids by this treatment are insoluble in acetone of low water content and therefore are not extracted with the plastid pigments, but remain in the solids. The slight quantity present in the ether-acetone solution of the pigments is washed out by the first few water washes used to remove the acetone from the ether.

No sodium carbonate should be added to the leaf tissues at the time of grinding if it is desired to measure the altered chlorophyll fraction. The altered chlorophyll is soluble in dilute alkaline solutions and will be re-

moved by the wash water used to wash the acetone from the ether-acetone solution of the pigments, if an excess of sodium carbonate is added to the leaf tissues at the time of grinding.

Both the grinding and the extraction of the sample are facilitated by the addition of acetone in 10-cc. quantities until a total of 50 cc. has been added. The addition of more than 50 cc. of pure acetone prior to the transfer of the sample to the Büchner funnel is of no advantage and is wasteful of solvents. The use of much less than 50 cc., however, is inadvisable because of the diluting effect of the water in the leaf tissues. If too little acetone is used a pasty mixture results which if drawn down on the filter with suction is difficult to extract. A more serious complication, however, is due to the fact that some of the substances in the leaf tissues; which are soluble in dilute acetone, are carried down into the suction flask and are thrown out of solution as a fine flocculate when more acetone is added. The presence of solids in the ether-acetone solution of the pigments and especially the presence of this fine flocculate increases the difficulty of washing out the acetone without the loss of some of the plastid pigments.

Redistilled acetone of low water content (85–95 per cent.) may be used instead of pure acetone to extract most of the plastid pigments, but enough should be added before transferring to the Büchner funnel to insure a concentration of at least 80 per cent. Pure acetone should always be used in the final washes, however, in order to dehydrate the solids before extraction with ethyl ether.

In order to obtain the altered chlorophyll fraction for estimation, the ether-acetone solution of the pigments is transferred to a 500-cc. separatory funnel and washed several times with pure distilled water to remove the acetone. The final washings should be free of flavones. The ether solution of the pigments is then washed very thoroughly with 50 cc. of dilute alcoholic KOH solution made by adding 10 cc. of methyl alcohol saturated with KOH to 1,000 cc. of water. Practically all of the altered fraction will be removed by the first wash with dilute KOH solution if the operation is properly performed. The layered solutions should be shaken together vigorously but for periods of short duration only. Prolonged violent agitation invariably results in the formation of emulsions. If emulsions form allow the solution to stand until both the aqueous and the ether layers have cleared. Finally, run the greenish KOH solution of the altered chlorophyll through a filter-paper into a 100-cc. volumetric flask. Wash the ether extract several times with small quantities of dilute KOH solution. Make the combined extracts up to volume with dilute KOH solution and estimate colorimetrically. This should be done very soon after extraction as the altered chlorophyll is very unstable. Keep the sample in a dark place until measured.

The altered chlorophyll fraction obtained by the above method from tobacco tissues is sufficiently free from other colored substances to permit its use in comparative studies.

Whether or not it is desired to measure the altered chlorophyll it should be removed prior to the saponification of the unaltered chlorophyll because of its effect in changing the color tone of the chlorophyllin solutions. It may be removed either with dilute KOH solution or with one per cent. sodium carbonate solution as recommended by SCHERTZ for the removal of the last traces of the flavones.

The amount of KOH necessary to saponify the chlorophyll is conditioned to a large extent by the volume of ether in which the pigments are dissolved. The greater the volume of ether the more KOH is needed to effect speedy saponification. The use of large quantities of KOH is to be avoided, however, because of the increased difficulty of extracting with ether the last traces of the yellow pigments from the strongly alkaline solution of chlorophyllin. In order to reduce the amount of KOH needed for saponification, ether should be used sparingly in the early stages of extraction. The amount used should not exceed 150 cc. Some of this will be lost in the process of washing out the acetone. A further reduction in the volume of ether may be effected by saponifying the chlorophyll directly in the separatory funnel instead of transferring it first to a bottle or flask. The above expedient eliminates, at the same time, several operations in the process of pigment separation.

After the removal of the chlorophyllin, the ether solution of carotin and xanthophyll should be thoroughly dehydrated by passing it through anhydrous sodium sulphate. Failure to dehydrate this solution before evaporating off the ether may result in the formation of emulsions when petroleum ether is added to redissolve the residue.

Transfer the residue from the distilling flask to a 500-cc. separatory funnel with a minimum quantity of petroleum ether and 75 cc. of 85 per cent. methyl alcohol.

Immerse the separatory funnel containing the petroleum ether-methyl alcohol fractions of the yellow pigments in a water bath at 45° C. and drive off the petroleum ether by means of a gentle stream of air. The force of the air should not be strong enough to ripple the surface of the liquid. When all but a thin layer of the petroleum ether has been evaporated off, remove the separatory funnel from the water bath and drive off the remaining petroleum ether while gently rotating the solutions in the funnel. The carotin will collect on the sides of the funnel and float on the surface of the methyl alcohol in thin flakes. Redissolve the carotin with 5 to 7 cc. of petroleum ether and drive off as above while gently rotating the solutions.

If the operation is properly performed none of the carotin flakes will be carried down into the methyl alcohol solution of the xanthophyll.

Run the methyl alcohol solution·of xanthophyll through a tight filter paper into a 200-cc. volumetric flask. If the alcohol layer is removed slowly, the carotin flakes floating on the surface will collect on the walls of the funnel. The filter paper will catch the occasional small particle that may be carried down.

Dissolve the carotin in the separatory funnel with 5 cc. of petroleum ether. Add 50 cc. of 85 per cent. methyl alcohol by gently flowing it down the sides of the funnel. Without using the water bath drive off the petroleum ether layer with air while gently rotating the solutions in the funnel. Run off the methyl alcohol as before, filtering it directly into the 200-cc. volumetric flask. Repeat the above operation using 5 cc. of petroleum ether and 25 cc. of 85 per cent. methyl alcohol. Frequently the alcohol in this extraction remains clear or is only faintly tinted with yellow. In this case no further extractions are necessary. If the alcohol from this wash is distinctly yellow, however, repeat the last operation.

The combined methyl alcohol extracts of xanthophyll are made up to 200-cc. volume with 85 per cent. methyl alcohol for estimation colorimetrically.

The alcoholic solution of xanthophyll obtained by the above method of separation is always clear. There is no necessity of transferring the xanthophyll to ethyl ether for estimation. This latter step therefore is omitted.

The filter-paper used in filtering the xanthophyll solution is dried in front of a fan at room temperature without being removed from its funnel. The carotin is dissolved from the walls of the separatory funnel with petroleum ether and run through the dried filter into a 100-cc. volumetric flask. It is made up to 100-cc. volume with petroleum ether for estimation.

In the above method of separating the carotin and the xanthophyll the danger of emulsion formation is eliminated, ·and, as compared to other methods, the time necessary for making the separation is greatly reduced. The accuracy of the above methods compares favorably with the longer and more difficult method of WILLSTÄTTER and STOLL as modified by SCHERTZ. It is important, however, if accuracy is to be achieved by the new method, to keep the concentration of the methyl alcohol at or slightly below 85 per cent. Higher concentrations may remove some of the carotin with the xanthophyll. Concentrations of 90 per cent. or above dissolve carotin rather readily after the complete removal of the petroleum ether. When this happens the carotin fraction will be low and the xanthophyll fraction correspondingly high.

In a recent paper by SPRAGUE and SHIVE (2) the authors suggest the substitution of petroleum ether for diethyl ether as recommended by

SCHERTZ as a solvent for the carotinoids. The substitution eliminates the necessity of driving off the ethyl ether after the separation of the green from the yellow pigments. In the writer's experiments, however, this short cut has always resulted in low and erratic yields of xanthophyll. Part of the xanthophyll is carried down with the chlorophyllin. Repeated washings with petroleum ether fail to remove the xanthophyll although it can be extracted easily by a single wash with ethyl ether. Another objection to the use of petroleum ether is the increased difficulty of preventing the formation of emulsions thus making every step in the separation with petroleum ether slower than the corresponding step with ethyl ether.

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    WASHINGTON, D. C.

LITERATURE CITED

1. SCHERTZ, F. M.   The extraction and separation of chlorophyll $(\alpha + \beta)$ carotin and xanthophyll in fresh green leaves, preliminary to their quantitative determination.   Plant Physiol. **3**: 211–216. 1928.

2. SPRAGUE, H. B., and SHIVE, J. W.   A study of the relations between chloroplast pigments and dry weight of tops in dent corn.   Plant Physiol. **4**: 165–192.   1929.

3. WILLSTÄTTER, RICHARD, and STOLL, ARTHUR.   Investigations on chlorophyll.   English translation by SCHERTZ, F. M., and MERZ, A. R. The Science Press Printing Company.   Lancaster, Pennsylvania. 1928.

SOME GROWTH CURVES OF BARLEY KERNELS[1]

MARY L. MARTINI, H. V. HARLAN, AND MERRITT N. POPE

(WITH EIGHT FIGURES)

Introduction

In the years 1915 to 1920, Harlan, Anthony, and Pope conducted an extensive series of kernel studies, mostly at Aberdeen, Idaho. In these studies the daily growth was determined by weighing individual kernels from spikes of a definite age. The dry weights were obtained for the kernels from the same spikes. These results have been published in part only.[2] In the present paper the writers have made use of both published and unpublished material. It was thought that a series of fitted curves might present a trend of growth in a light sufficiently different from that of the previous treatments to justify its presentation.

Methods of procedure

The simplest logarithmic curve, $y = a + b (\log x)$, represents the general trend of most growth observations. A number of variants of this curve were tried in attempting to fit a curve to these data. The one finally used, $y = a + bx + cx^2 + d (\log x)$, gives an unusually good fit except for the first few days. For all varieties, with but one exception, Chevalier, this formula depresses the curve below the actual weights in these earlier days. In some instances this depression extends below the zero line.

It should be clearly understood that these curves are not prediction curves in any sense of the word. As stated above, these curves are roughly the growth curve common to all growing things. However, there are some very essential differences.

Up to the time of seed formation the barley plant is undergoing typical growth. Each day the added leaf and root tissue are functional and this accounts for the acceleration which is often likened to the acceleration of

[1] Contribution from the Office of Cereal Crops and Diseases, U. S. Department of Agriculture.

[2] HARLAN, H. V. Daily development of kernels of Hannchen barley from flowering to maturity at Aberdeen, Idaho. Jour. Agr. Res. **19**: 393–430. 1920.

———, and ANTHONY, S. B. Development of barley kernels in normal and clipped spikes and the limitations of awnless and hooded varieties. Jour. Agr. Res. **19**: 431–472. 1920.

———, ———. Effect of time of irrigation on kernel development of barley. Jour. Agr. Res. **21**: 29–45. 1921.

———, and POPE, M. N. Water content of barley kernels during growth and maturation. Jour. Agr. Res. **23**: 333–360. 1923.

compound interest or of catalytic activity. When seed formation starts, however, another factor must be considered. The addition of leaf and culm tissue diminishes rapidly and ceases entirely after a few days. From this time until near maturity the parts of the plant functional in producing elaborated food for storage do not increase. The seed theoretically is able to store the daily intake. Near maturity the leaves normally commence to mature and the functional parts thus undergo a daily reduction.
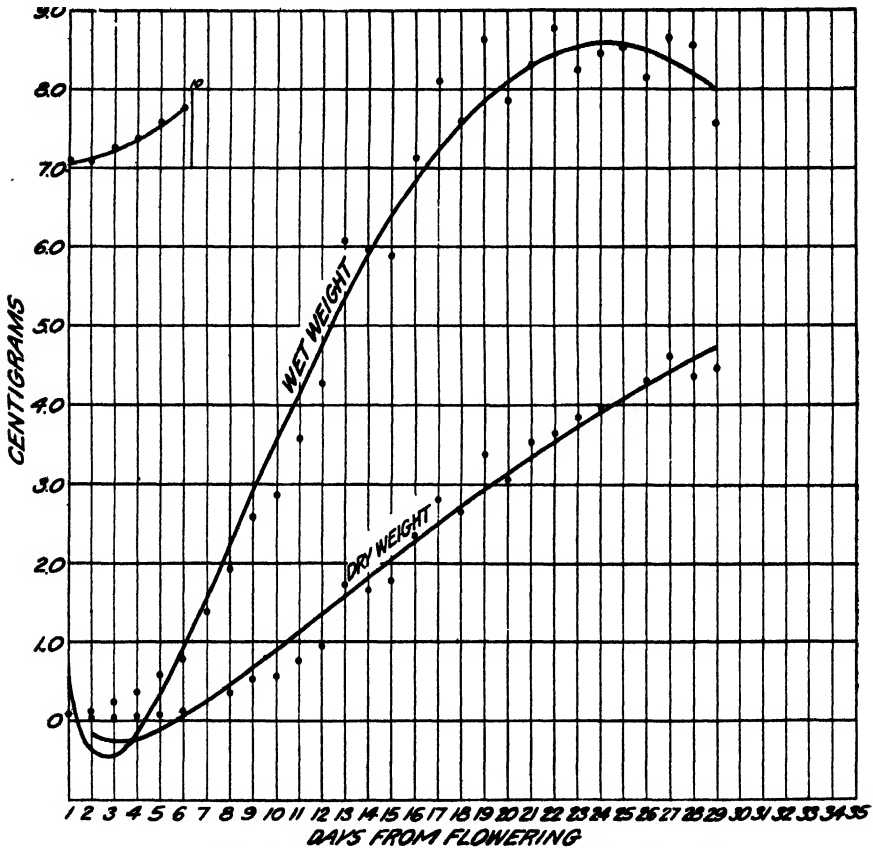
FIG. 1.   Deficiens, with inset of first six days fitted independently.

The kernel itself, for a short period, undergoes development of a true growth nature. The cells of the endosperm are rapidly increased in number and the total rate of function of storage cells accelerated. By the time the growth of the culms and leaves is completed the number of storage cells has become nearly constant and from then until near maturity there is probably little change in the daily capacity of the kernels to store food, although some cells are added near the crease. During the main period of deposit

the dry matter curve is a nearly straight line. As maturity approaches there is less food to store, there are fewer cells of the kernel active, and, as far as these curves are concerned, there are individual kernels appearing in the averages which have ceased to function. Since the kernels mature one at a time, the acceleration in averages is modified considerably.

Discussion

The graphs tell the story of rapid or slow deposit as affected by variety more clearly than it can be told in words. It is unfortunate that the data on the hulled varieties could not be obtained for a longer period. The adherence of the hull after the kernel starts to dry makes this impossible. While the curves are not predicting curves, one can visualize by analogy with the naked varieties the probable after-trend in the hulled sorts. In the naked varieties there is no obstacle to prevent carrying the weights to full field dryness.

In figure 1 the growth curve of *deficiens* for 1918 is presented. The kernels of *deficiens* grow more rapidly and the final dry weight is greater than that of Hannchen which has been used in earlier publications. In this variety the formula used causes a very marked inaccuracy of fit in the first six days of the growth period. In both the wet and the dry weight the curve is depressed below zero and that of the wet weight depressed below that of the dry weight, although the kernels at this stage contain nearly 80 per cent. of water. This is purely a mathematical result. The curve of wet weight is more complicated than that of dry weight, as it contains one additional factor, water. If the same formula is used and the results based on the first 6 days only, this error is corrected. (See insert on figure 1.)

If the first 6 days are omitted in the wet weight, the fit for the succeeding period is much better than when the entire data are used. This period of *deficiens* is illustrated in figure 2. This can not but mean that the "S" curve of growth is not wholly applicable to the development of barley kernels. There evidently are more factors to be considered than mere growth. The lack of fit in the first 6 days is undoubtedly due to the fact that the kernel weights represent a number of factors. In the early stages when each cell of the endosperm and embryo is dividing, kernel growth acceleration probably is taking place. During this period the cells of the kernel probably are not capable of utilizing all of the food manufactured by the leaves and gathered by the roots and moreover some parts of the plant are still actively growing so that some of this food is being used elsewhere. Before the tenth day the food-gathering area and leaf surface have become stabilized and the entire output is deposited in the kernels. This results in a reasonably uniform daily deposit up to when signs of maturity appear.

As maturity approaches parts of the leaves cease to function and some of the cells in the kernels cease to be active in the deposition of starch. The final stages of maturity, as far as these curves are concerned, also are influenced by the fact that the kernels mature one at a time. Thus the averages on which the daily increment is based include an increasing number of kernels which have ceased to function.
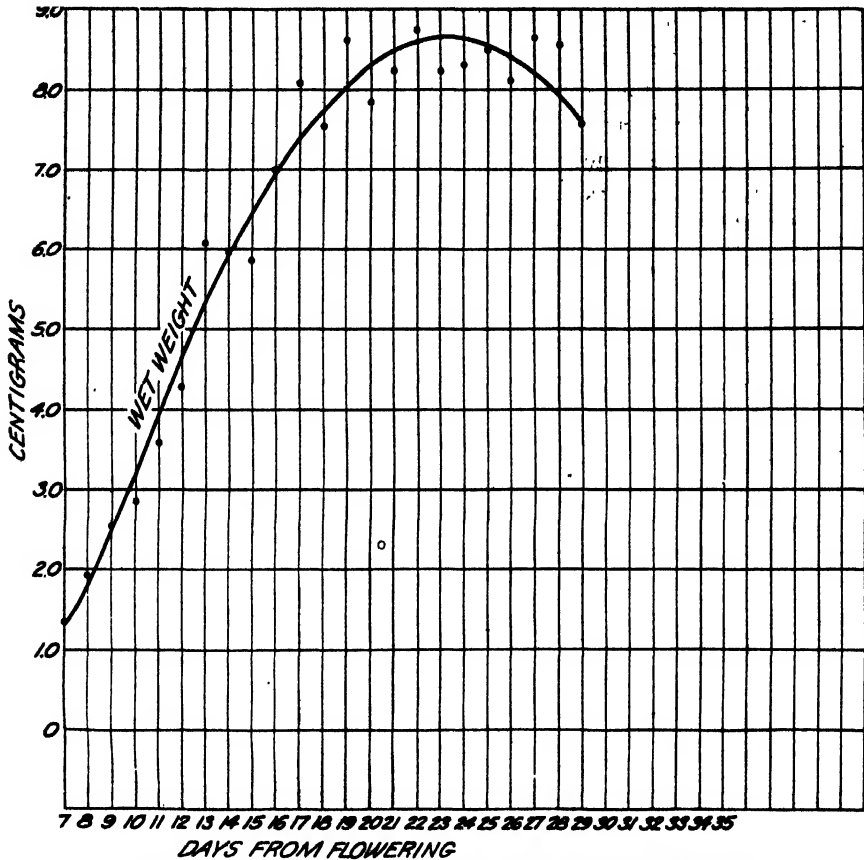
FIG. 2. Deficiens wet weight from seventh day to maturity.

This brings us back again to the formula. In order to secure a fit it was necessary to add another factor, C. What is the significance of C? Since it results in a fit of the data from 6 days to maturity, it must take care of a factor that is operating throughout this period. One significant and major factor in the growth of the kernel is the decrease of the percentage of water. This continues from the day of flowering until maturity, accelerating rapidly towards the end. Since the total water increases until near maturity and then falls rapidly, water might be considered first as a posi-

tive factor and then as a negative one. On the other hand, if the water increased proportionately with the dry matter, the daily growth would be much greater, and from this standpoint water acts as an inhibiter of full theoretical increment from the beginning. C is always minus in these data. It may, at least partially, thus take care of the difference between the 80 per cent. of water at flowering and the actual content on the successive days.

It is obvious that similar influences are operating in the curves of wet
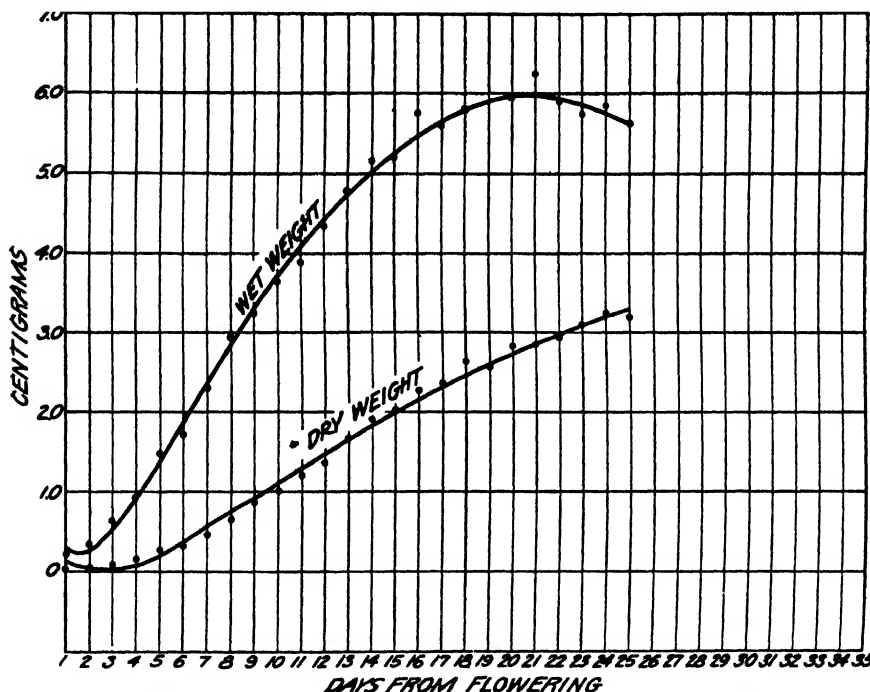
FIG. 3. Hannchen.

weight in other varieties and that they are susceptible of similar treatment. The date upon which the first curve should terminate varies, however, and it is thought that the date is more apparent to the reader if the curves are not altered.

Curves have been fitted to the data on 8 varieties. Figure 3 illustrates the results obtained from Hannchen in 1917. This variety is presented because it has been used in the earlier publications as a basis of kernel studies. It will be noted that the curve of the wet weight is still actively on the downward trend at the time the kernels had dried to the place where sampling was no longer possible. Hannchen is a small-kerneled variety as compared with other 2-rowed sorts and, indeed, as compared with the central kernels of the two 6-rowed sorts included in the 8 varieties.

In figure 4 the central kernels of Trebi barley were used as a basis. It is obvious that in this case the rate of deposit of dry matter had not yet been checked when sampling ceased. Indeed, the curve of wet weight had not yet turned downward. The large daily increment and the long continued activity doubtless are associated with the high yielding capacity of this variety. Even at the time sampling had ceased the dry weights were around 48 milligrams.
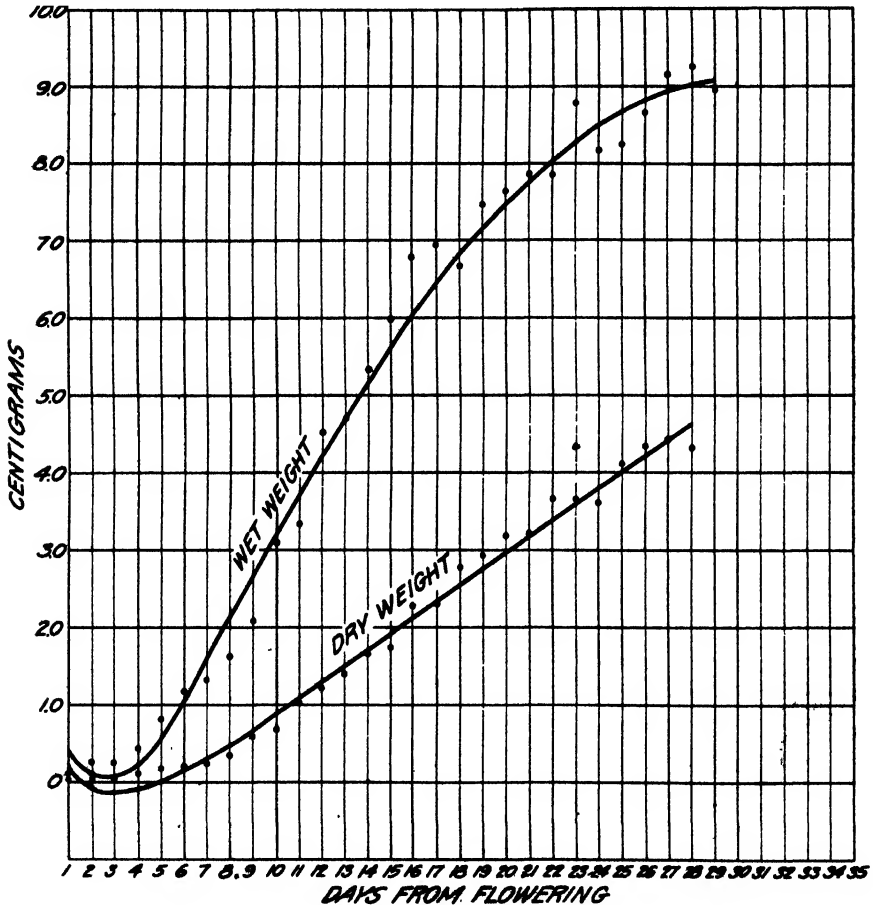
FIG. 4. Trebi.

Although there is no water in the dry weight, the formula containing C fits better than the same formula without it. The dry weights, however, are so nearly a straight line that no curve formula tried is wholly satisfactory with them.

Figure 5 represents the growth of Meloy, a hooded 6-rowed variety. Here again only the central kernels were used. The daily increase was

much less than in the case of Trebi and the curve of wet weight was markedly on the downward trend at the time sampling was stopped. The hooded varieties usually do not yield as well as the awned sorts, but Meloy is among the best of the hooded kinds. In most hooded sorts occasional kernels commence to ripen when most of the spike is still quite active. This, of course, would result in a lower average weight at full maturity.
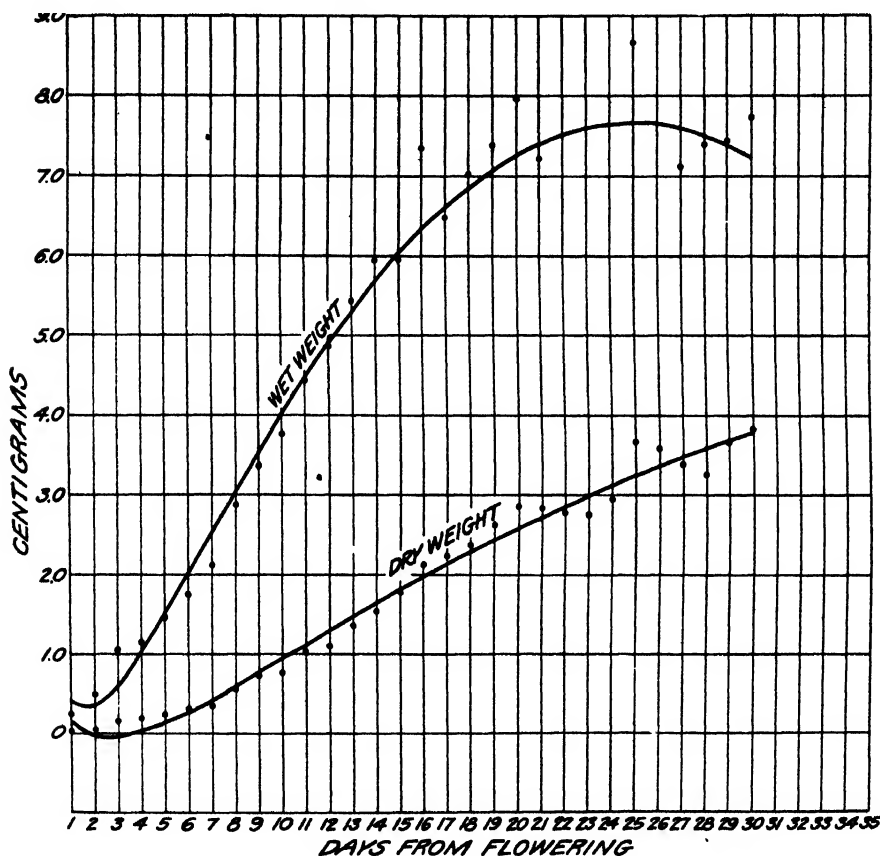
FIG. 5. Meloy.

Although naked varieties are not commercially important, they are well adapted to kernel studies. In the naked sorts sampling can be continued until the kernels are as dry as they will become in the field. A growth curve of Baku, a naked 2-rowed sort, is shown in figure 6. The original observations are included in the graph so as to show the relation of the fitted curve to the data. With the exception of the first days, the fit is all that could be desired. When sampling is continued to complete dryness, the wet and dry weights approach each other quite closely.

An interesting feature of figure 6 is the immense pyramid of water
which is included in the actively growing kernels. Of course, the area be-
tween the curve of dry weight and that of wet weight represents water.
This water, while possibly not truly functional, must be necessary for the
accomplishment of the dry weight deposits.
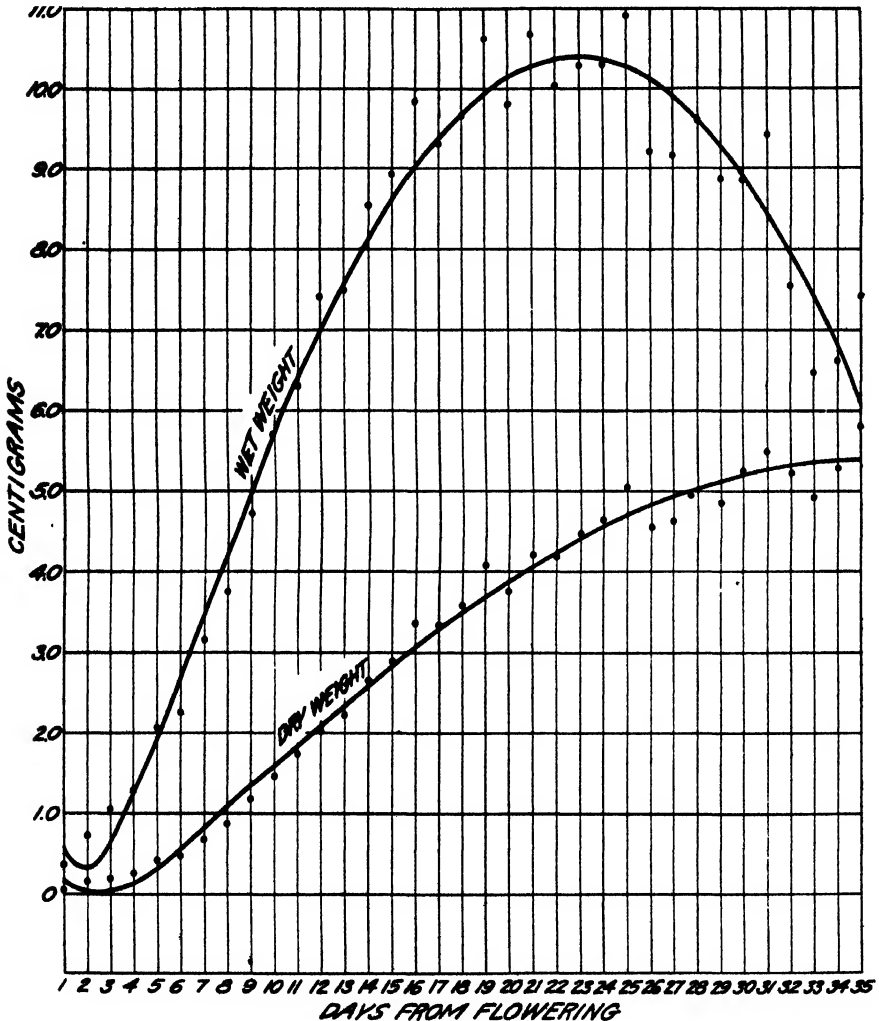
FIG. 6. Baku.

This period is even more striking in the curves of figure 7, which por-
trays the results obtained with Jet, another 2-rowed naked barley. The
sharpness of the peak of wet weight in this case is due to two factors. The
growing period is not so long as with Baku, which naturally results in a

more rapid decline after the peak is reached. The ratio of water to dry weight at the peak is a little higher in the case of Jet. This is probably due to the fact that this variety is possessed of a thickening of the tissue on the dorsal surface of the kernel outside the aleurone. This thickening runs lengthwise of the kernel directly opposite the crease. Its function is not known.
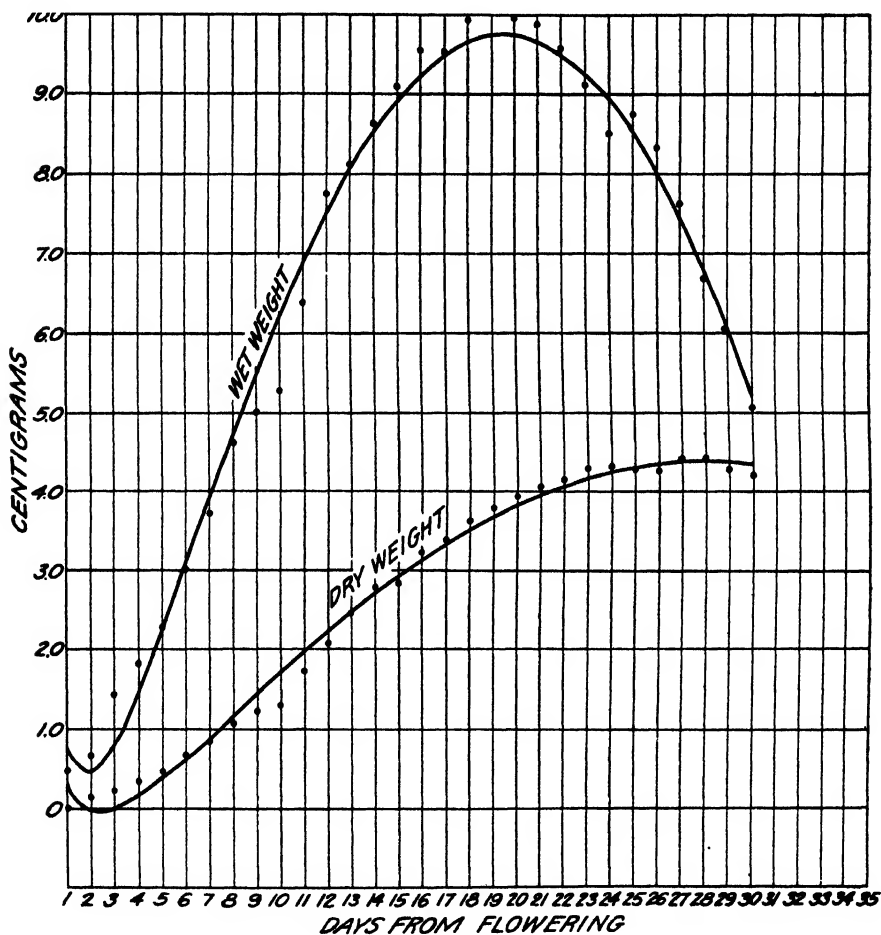
FIG. 7. Jet.

The wet weights of two 2-rowed varieties, White Smyrna and Chevalier, are given in figure 8. The dry weights of these varieties are not available. White Smyrna produces the largest kernels of any variety in our collection. The curve of wet weight is, therefore, steeply inclined and decidedly elevated at its peak. The full size of the kernel was not yet reached when sampling ceased.

In Chevalier the usual mathematical depression is not apparent at the beginning. The exact reason for this is not clear. Either the first samples were collected at a later stage than in the other varieties or they started growing with greater rapidity. It is likely that the kernels were older
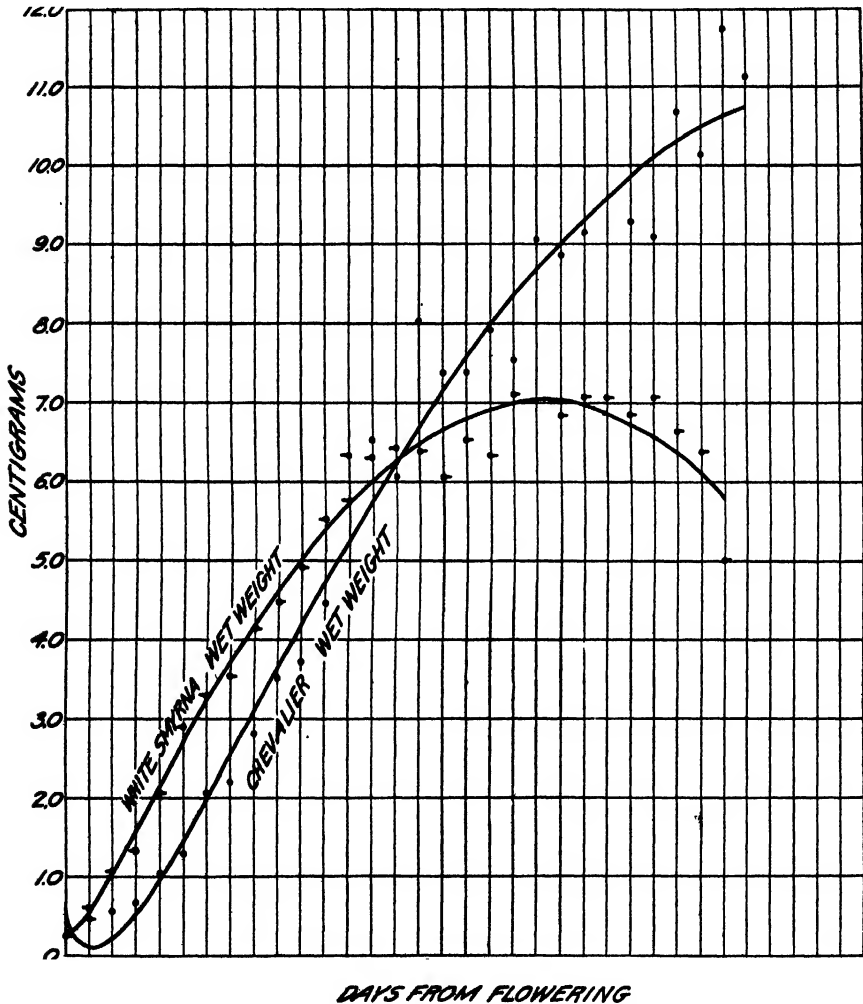
FIG. 8. White Smyrna and Chevalier.

when the sampling started. Spikes of identical age are most easily obtained by tagging them as the awns appear above the sheath. It may be that the spikes of Chevalier were more advanced than other varieties when the awns emerged and the time of flowering not accurately determined.

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A CASE OF SECOND CROP SEEDLESS APPLES[1]

C. B. WIGGANS

(WITH THREE FIGURES)

During the summer of 1925 a large number of second bloom apples were produced in the University of Arkansas Yellow Transparent orchard. Upon cutting some of these fruits in cross-section the writer was surprised to find them seedless. It seemed at first to be a case of parthenocarpy, but upon further study the evidence pointed to embryo abortion (4).

Mature apples normally have from a few to ten fully developed seeds. Various investigators have found a close correlation between the number of normal seeds and the setting and development of various fruits. SAX (7) found seed content to be correlated with setting and regularity in shape of apples, while MÜLLER-THURGAU (6) found size of grape berries to be closely related to seed content. The fact that lop-sided fruits are generally accompanied by the absence of seeds on the less developed side, as pointed out by

TABLE I

EFFECT OF SECOND BLOOM ON PERCENTAGE OF ABORTED OVULES IN YELLOW TRANSPARENT APPLES, 1925

DIAMETER SIZE OF FRUIT	NUMBER OF NORMAL OVULES	TOTAL FRUITS WITH ALL OVULES ABORTED	
mm.		no.	per cent.
7.5–10.0	0	11	100
10.0–12.5	0	47	100
12.5–15.0	0	40	100
15.0–20.0	2	56	97
20.0–25.0	0	72	100
25.0–30.0	1	36	97

WICKS (10) and others, is further evidence of the importance of normal seed development in connection with normal growth of the apple fruit. The cause of this uneven development of seeds may be due to improper pollination, abnormal ovules, unfavorable weather during or following pollination, or some other cause.

Although few of the fruits attained a greater size than 30 mm. in diameter and were, for the most part, malformed, many remained on the trees until frost. When over 250 of these fruits of various sizes were cut open, all were found to be without normal seeds except three. The three excep-

[1] Research paper no. 182, Journal Series, University of Arkansas.

tions had one normal seed each (table I). VINSON (8) reports a second
crop of blossoms on Smokehouse, Rambo, and other varieties following frost
destruction to the first crop but did not report on seed development in the
second crop fruit.
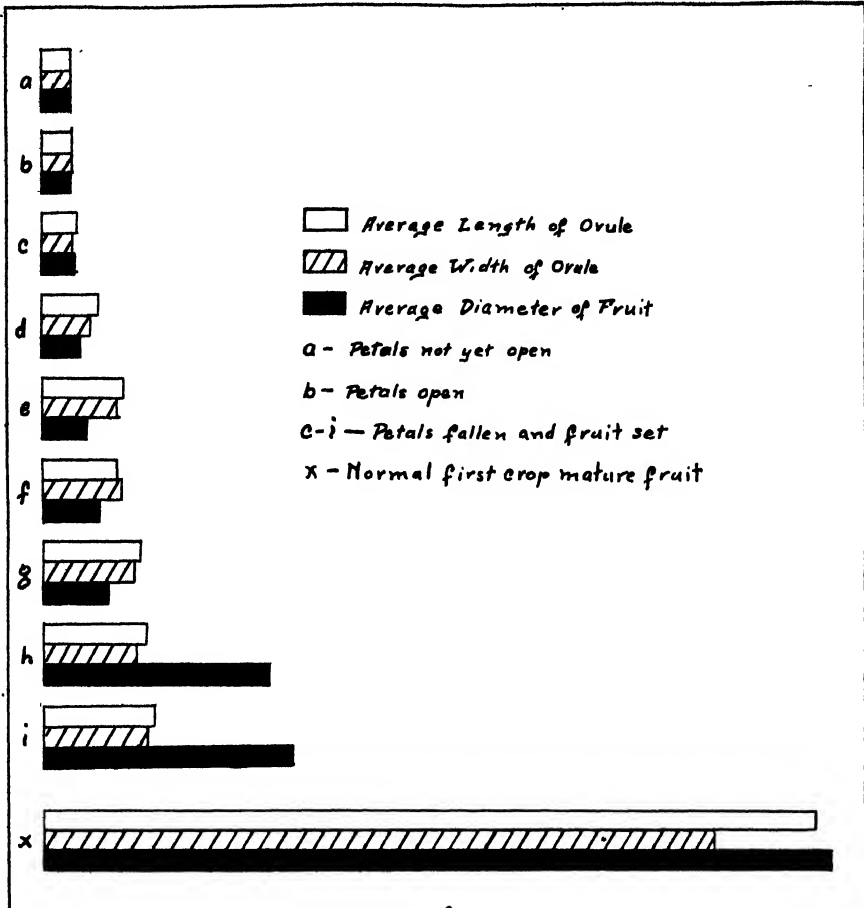
FIG. 1. Graph showing the relative progressive development of the ovules or unde-
veloped seeds and pericarp in second crop Yellow Transparent apples, and the approxi-
mate size of the same in. normally developed first crop apples. All are expressed in
percentage of size of specimen before blooming. Note the enlargement of ovules fol-
lowing blooming (e, f, and g), and the decided slowing up thereafter (h, and i).

The seedless condition, described above, was not parthenocarpic. This
was borne out when, upon measuring the ovules and aborted immature or
rudimentary seeds of the various sized fruits, it was found that there was
a decided enlargement shortly following the fall of the petals. The appar-
ently fertilized ovules rapidly increased in size, more rapidly than did the

pericarp for considerable time, but gradually ceased growing (table II and figure 1).

The ovules, under the microscope, appeared normal before petal fall, but shortly after that stage was reached they appeared shrunken and shriveled. Finally in fruits 30 mm. in diameter or larger, the ovules were brown and
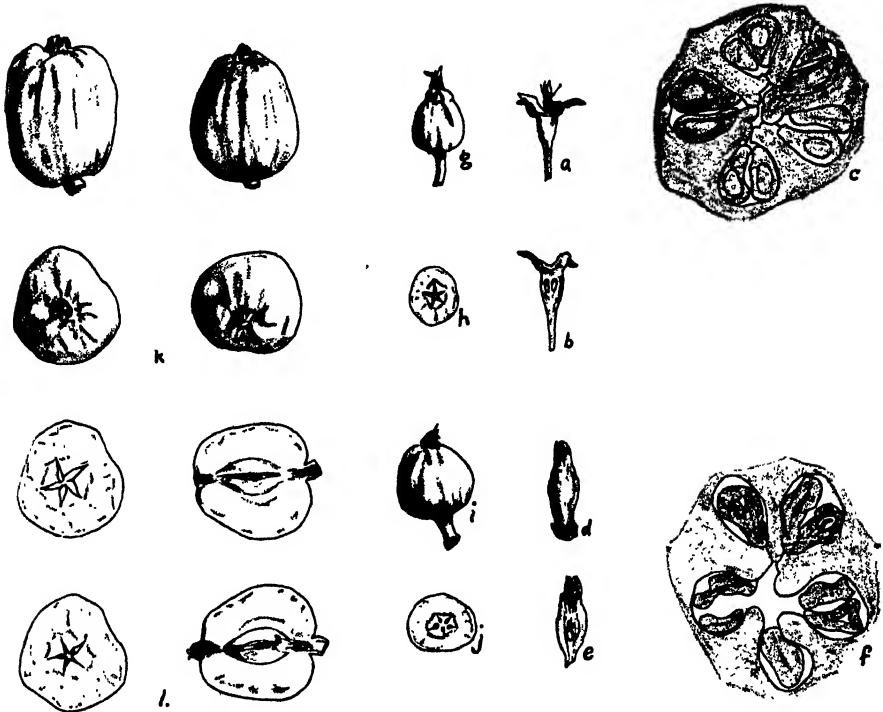
FIG. 2. Sketches of second crop Yellow Transparent at various stages of development.

A–B–C, immediately following petal fall showing ovules apparently normal.

D–E–F, a slightly later stage showing the somewhat larger, but already aborting embryos.

G–H, and I–J, successive stages showing the enlarging fruits with young seeds developed little, if any, more than in D, E, and F.

K, exterior appearance of seedless fruits.

L, the longitudinal and cross-sections of the same fruits as shown in K. Note the minute shriveled aborted seeds which have long since ceased to function.

were found with difficulty (figures 2 and 3). This is taken as proof that fertilization had been accomplished, but embryo abortion (4) occurred causing the seedless fruits.

By defoliating a Yellow Transparent and a Ben Davis tree in early summer, 1926, an attempt was made to force them into a second blossoming.

TABLE II

OVULE GROWTH COMPARED WITH FRUIT GROWTH, SECOND CROP YELLOW
TRANSPARENT, 1925

STAGE OF DEVELOPMENT	FRUIT, AVERAGE DIAMETER	OVULES	
		LENGTH	WIDTH
	mm.	*mm.*	*mm.*
Petals not yet open	2.7	0.414	0.252
Flowers open	2.7	0.427	0.260
Petals fallen-fruit set.................	3.0	0.483	0.267
" " " "	3.5	0.791	0.418
" " " "	4.0	1.122	0.633
" " " "	5.0	1.050	0.668
" " " "	6.3	1.367	0.767
" " " "	20.0	1.435	0.792
" " " "	22.0	1.555	0.868

The Transparent tree responded rather well but not as well as expected. Only a few blossoms appeared on the Ben Davis. From this limited trial it seems that Transparent trees can be forced from the rest period more readily than the Ben Davis. The observations on the limited amount of material obtained in 1926 were substantially the same as in 1925.

In 1929 in spite of the extreme drought and the great loss of foliage due to disease early in the season little second bloom was observed in Northwest Arkansas and the writer was able to secure only four second crop fruits, and
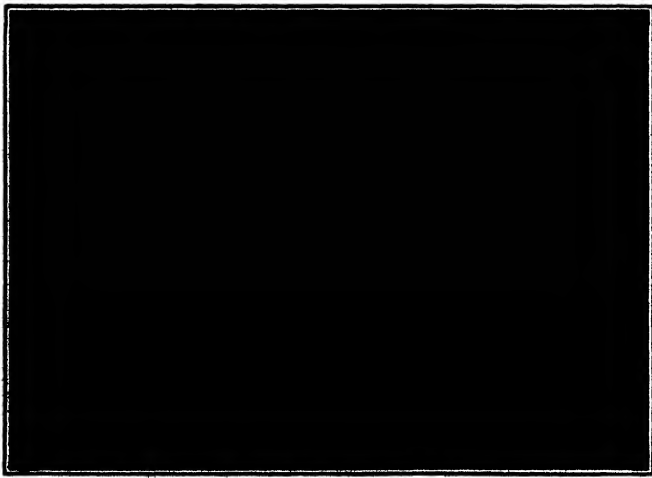
FIG. 3. Abnormal second crop Yellow Transparent apples. Only aborted seeds can be distinguished where mature seeds should be.

these were Coffelts. These fruits were one-half inch or less in diameter and were obtained after a severe frost had occurred so that the true condition of the young seeds could not be definitely established. Some of them appeared, however, to be more nearly normal than those described above. These ovules might have aborted at a later stage, although having bloomed under cooler weather condition, it is possible that they would have developed normally. It seems likely that the high temperatures during the second blossoming in 1925 and 1926 contributed largely to the abnormal development described.

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LITERATURE CITED

1. BROWN, F. R. Seedless and malformed fruits. First Bienn. Crop Pest and Hort. Rept. Oregon Agr. Exp. Sta. 41–43. 1911–12.
2. CHANDLER, W. H. Fruit Growing. P. 177. Houghton Mifflin Co. 1925.
3. COOPER, J. R. Unpublished data. Arkansas Exp. Sta.
4. GARDNER, V. R., BRADFORD, F. C., and HOOKER, H. D. The fundamentals of fruit production. McGraw-Hill Book Company. 1922.
5. MARSHALL, R. E. Report of three years' results in plum pollination in Oregon. Proc. Amer. Soc. Hort. Sci. **16**: 42–49. 1919.
6. MÜLLER-THURGAU, H. Seedless grapes and orchard fruit. (Translated title.) Landw. Jahrb. Schweiz. 22: 564–597. 1908.
7. SAX, KARL. Studies in orchard management.—II. Factors influencing fruit development of the apple. Maine Agr. Exp. Sta. Bull. 298: 53–84. 1921.
8. STOUT, A. B. Types of flowers and intersexes in grapes with reference to fruit development. New York Agr. Exp. Sta. Tech. Bull. 82. 1921.
9. VINSON, C. G. Secondary flowering of the apple. Proc. Amer. Soc. Hort. Sci. **19**: 173–179. 1922.
10. WICKS, W. H. The effect of cross-pollination on size, color, shape, and quality of the apple. Arkansas Exp. Sta. Bull. 143. 1918.

THE MASS FACTOR IN THE ENERGY RELATIONS OF LEAVES

CHARLES A. SHULL

In a recent paper the writer (4) emphasized the variability of the reflection factor in the energy relations of leaves, and pointed out the fact that reflection is frequently large, and not to be neglected in determining the absorption coefficient as was done by BROWN and ESCOMBE (1, pp. 75 and 91) in their fundamental studies of the energetics of the leaf. There is evident need of a reinvestigation of the problems of energy exchange between the leaf and its environment, and the internal transformations of energy in the processes of transpiration and photosynthesis, with due regard to the variability of the reflection and absorption of energy in all regions of the spectrum, from the far infra-red to the limits of the solar ultra-violet. Attention is called to an error in the previous paper (4, p. 604) which states that the BROWN and ESCOMBE studies were made "only on *Catalpa bignonioides.*" While some of their important measurements were made on the hypostomatous leaves of this species, in several sections of their work numerous species were used for studies of photosynthesis, thermal emissivity, and respiration.

The equation used by BROWN and ESCOMBE to calculate the heating effects of the absorbed radiation, on the supposition that there were no dissipation of energy by internal work, is $Ra/ms = t$ in degrees C. rise per minute, in which R is the total incident radiation per square cm. per minute, a the coefficient of absorption of energy, m the mass of the square centimeter of leaf lamina, and s the specific heat of the leaf substance. The values substituted for these symbols were as follows: $R = 0.8$ calorie, $a = 0.78$, $m = 0.020$ gm., and $s = 0.879$. The values used for m and s were obtained from measurements made on the leaves of *Helianthus annuus*. The value used for a is larger than that found for *H. annuus,* which was 0.687 as the average of three determinations, instead of 0.78; and the value assigned to R is also greater than those obtained during the work on *Helianthus,* which varied from 0.591 to 0.636 calorie per square cm. per minute, but less than the highest values observed on very clear days, which were from 0.932 to 1.019 calories. The authors state, however, that the mean results of observations extending over several hours were seldom over 0.550 calorie per square centimeter per minute, even on the clearest day. So the values assigned to the quantities in the numerator of the fraction in the equation are both relatively high.

It is obvious that the value of the denominator will vary either with the mass of the leaf per square centimeter, or with the specific heat. As most

leaves are fairly high in water content, the specific heat is probably much less variable than the mass factor. The latter is quite variable, and the value chosen by BROWN and ESCOMBE applies to a large group of thin-leaved plants, but does not apply very well to coarse textured or succulent leaves. In order to have a more general picture of the thermal effects of restricted internal dissipation of energy, some measurements of the mass factor have been made on a variable group of species which cover the range of possibilities pretty well.

If one uses the values assigned by BROWN and ESCOMBE to these various factors to calculate the rate of rise of temperature in a leaf in which no internal transformations of energy were occurring, the rise in temperature is about 35.5° C. per minute; but in the case of the heavy textured leaves, and especially with the succulent leaves, the temperature rise with zero dissipation of absorbed energy would be much less. In the thinnest leaves it is considerably higher than their estimate.

The data obtained in these measurements are given in the accompanying table I, and the thermal effects of energy absorption without energy dispersal are given in the final column of the table. In making these calculations, BROWN and ESCOMBE's values for R, a, and s have been used.

With only a few exceptions, the data of the middle column of the table are the average weights of 10 discs, each one square centimeter in area, the leaves having been cut with the Ganong leaf punch. In the exceptional cases the tissues were too thick for the punch, and pieces 1 cm. wide and 10 cm. long were cut accurately and weighed. In all cases the weights given are for 1 square centimeter of leaf tissue.

The heaviest leaf used was that of the aloe, whose thickest portions in the middle of the leaf weighed 69 times as much per square centimeter as the lightest leaves used (Galinsoga parviflora). Correspondingly, the rise in temperature per minute, on the basis of absorption of energy without dispersal, is approximately 0.75° per minute for the Aloe leaf, as compared to 51.8° for the leaves of Galinsoga.

If the value of R is taken as 0.55 calorie, which seems nearer the actual average energy receipt by plant leaves, and if the coefficient of absorption is corrected for the reflection, which averages about 10 per cent., the value of Ra will be greatly reduced. In addition the value of Ra should be reduced by the amount of energy used in photosynthesis, to obtain the quantity of energy to be dissipated in transpiration and thermal emissivity. The absorption coefficient found for Helianthus was 0.68, including the reflection, so that a value of 0.58 might be considered as near to the actual absorption. With these two modifications in the values, and subtraction of the energy used in synthesis, the energy to be dissipated is found to be only a

TABLE I

THE MASS FACTOR AND THERMAL EFFECTS OF ABSORBED RADIANT ENERGY IN THE ABSENCE
OF ENERGY DISPERSAL

SPECIES	MASS PER CM.^{2}	Ra/ms
	gm.	*degrees C.*
Galinsoga parviflora	0.0137	51.81
Sagittaria latifolia var.	0.0139	51.07
" " "	0.0193	36.78
Ambrosia trifida	0.0142	49.99
Viburnum opulis americanum	0.0146	48.62
Ailanthus glandulosa	0.0152	46.70
Ptelea trifoliata	0.0156	45.50
" "	0.0159	44.65
Catalpa bignonioides	0.0163	43.55
" "	0.0191	37.16
Sambucus canadensis	0.0167	42.51
Ulmus americana	0.0174	40.80
" "	0.0214	33.17
Panicum sp.	0.0177	40.11
Amaranthus retroflexus	0.0180	39.44
Cucurbita sp.	0.0181	39.22
Populus deltoides	0.0197	36.03
" "	0.0201	35.32
" "	0.0213	33.33
Taraxacum taraxacum	0.02016	35.21
Rhus cotina	0.02024	35.07
Helianthus annuus	0.0212	33.49
Clematis paniculata	0.0223	31.83
Vinca variegata	0.02596	27.35
Geranium sp.	0.02604	27.26
Ginkgo biloba	0.0268	26.49
" "	0.0278	25.53
Syringa vulgaris	0.0317	22.39
" "	0.0333	21.32
Impatiens balsamina	0.0379	18.73
Dioon spinulosum	0.0380	18.68
Ceratozamia mexicana	0.0407	17.44
Nelumbo lutea	0.0520	13.65
Iris germanica	0.0731	12.45
" "	0.0808	8.78
Agave americana	0.1524	4.66
" " (mid-rib)	0.8300	0.85
Crassula arborescens	0.3037	2.34
Aloe arborescens	0.9465	0.75

little over 50 per cent. of the value used in the calculations of table I. The rate of temperature rise for *Galinsoga* leaves would be only about 26° instead of 51.81° per minute, and for the *Aloe* leaf about 0.37° instead of 0.75° C.

The energy receipt, if dissipated only by thermal emissivity, would cause a rise in the leaf tissue of only 4.4°, $\left(\dfrac{0.55 \times 0.58}{2 \times 0.0366}\right)$, above the temperature of the surrounding air, according to the figures of BROWN and WILSON (2) for air flowing at a little over 5 miles per hour. The fact that much higher temperatures have been measured in the leaves of some succulents (3) is related to the lower transmission in such thick leaves, and to greater energy receipt from the sun in very arid regions. When thermal emissivity and transpiration work together in the dispersal of energy, the rise in temperature required to dissipate the entire receipt of radiant energy is considerably less, possibly not over half as much as when thermal emissivity works alone. In the work by BROWN and ESCOMBE (1, table VIII, pp. 100–103), the energy lost by thermal reradiation and convection was in some instances 5 or 6 times as great as that lost by water evaporation, while in other cases transpiration exceeded thermal emissivity as a means of energy dispersal by as great proportion. Averaging the results of 13 experiments by BROWN and ESCOMBE, transpiration is responsible for about 55 per cent. of the energy dispersal, thermal emissivity for about 45 per cent. It is fair to assume then, that under ordinary outdoor conditions, the rate of rise in temperature of leaves by direct insolation will be less than half of the values shown in the last column of table I, on the average, and the total rise relatively small as the transpiration becomes decidedly the predominating agent of energy loss.

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LITERATURE CITED

1. BROWN, HORACE T., and ESCOMBE, F. Researches on some of the physiological processes of green leaves, with special reference to the interchange of energy between the leaf and its surroundings. Proc. Roy. Soc. London B **76**: 29–111. 1905.

2. —————, and WILSON, W. E. On the thermal emissivity of a green leaf in still and moving air. Proc. Roy. Soc. London B **76**: 122–137. 1905.

3. PEARSON, W. H. H. Observations on the internal temperature of *Euphorbia virosa* and *Aloe dichotoma*. Ann. Bolus Herb. **1**: 41–66. 1914.

4. SHULL, CHARLES A. A spectrophotometric study of reflection of light from leaf surfaces. Bot. Gaz. **87**: 583–607. 1929.

JAN INGEN-HOUSZ
1730–1799

BRIEF PAPER

JAN INGEN–HOUSZ

JAN INGEN-HOUSZ (plate II) was primarily a physician, yet during the latter part of his life he became interested in the physiology of plants and made noteworthy contributions to this subject. His life and works have been extensively reviewed by Professor JULIUS WIESNER in a biography which was published at Vienna in 1905 under the title "JAN INGEN-HOUSZ, sein Leben und sein Wirken als Naturforscher und Artzt."

INGEN-HOUSZ was born December 8, 1730, in Breda, the head town of the province of Northern Brabant in the Netherlands. His father had come to Breda from Geldern, and JAN seems, therefore, not to have descended from the INGEN-HOUSZ family of North Brabant, famous in military affairs. The ancestry of JAN INGEN-HOUSZ is not known certainly beyond his grandfather.

Not much is known about the parents of INGEN-HOUSZ, but they had sufficient means and social position to give JAN an education far superior to that common to his time. His mother died when he was very young. He had a brother, LOUIS, who became an apothecary in Breda and from whom there descended a line of physicians.

At sixteen, INGEN-HOUSZ finished the Latin school in Breda, being remarkably proficient in Latin and Greek. The principal of the Breda gymnasium, HOOGEVEEN, prophesied a brilliant career for him.

He entered the University of Löwen to study medicine, and also became deeply interested in physics and chemistry. After receiving the degree of M.D. at the age of twenty-two, he went for further training to Leyden, to study under the famous teachers of medicine there, GAUBIUS and ALBINUS. After about two years there, he visited for a short time the Paris Medical School, and later the University of Edinburg.

In 1757 he came back to his home town of Breda and established a medical practice. He soon acquired a reputation for great ability, and his practice grew so large that it occupied all of his day time. He was obliged to carry on the scientific experiments in which he was so interested by night. He was particularly interested in frictional electricity, and perfected an electrical machine.

His successful medical practice and researches in physics built up a remarkable reputation for him in scientific circles. He kept up a widespread correspondence with his teachers in the universities which he had

attended, but was slow to publish the results of his experiments although his friends urged him to do so. This deliberation in publication is responsible for the maturity of his writings. His first publication appeared when he was twenty-eight years old.

. The Netherlands could offer him no position suitable for his abilities, a teaching position in the University being closed to him on account of his religion. Ample opportunities were offered him in other countries, but love of his aging father kept him in Breda as long as his father lived.

After the death of his father, INGEN-HOUSZ accepted the invitation of Sir JOHN PRINGLE and other English friends to go to London. PRINGLE, then the foremost physician in England, was an old friend of the INGEN-HOUSZ family and had always been interested in the talented young physician. This interest led to a lifetime intimacy and mutual esteem. PRINGLE's influence opened the highest medical and scientific circles of London to INGEN-HOUSZ. He especially profited by association with the two HUNTERS, with ARMSTRONG, and MONRO.

INGEN-HOUSZ also made a visit to Edinburgh, where he made a special study of the clinical work of the hospitals. On coming back to London, he absorbed himself in the study of the diseases of children, particularly in the vaccination of children for smallpox.

At this time in London, also, he began to publish both medical and scientific papers. In several of these he advocated vaccination and attacked the opponents of it. He kept a small amount of time for the researches in physics, which he loved, and for association with famous physicists in London. But the major part of his time and attention he now determined to devote to the furthering of the cause of vaccination to combat the ravages of smallpox. It was his work in this subject that led to his call to the court of the Empress of Austria, Maria Theresa, to vaccinate her children.

INGEN-HOUSZ left London the first of April, 1768, made a short visit to his home in Breda, and arrived in Vienna on the 14th of May. He was received everywhere with honor, for his fame was widespread. In Vienna his countryman, GERHARD VAN SWIETEN, greeted him and the Empress had made his reception quite royal, having prepared for him two houses, one near the palace, and one in the country, at Schönbrunn. A coach and servants also awaited his pleasure.

INGEN-HOUSZ became a person of the greatest fame and popularity in Vienna as a result of his successful vaccination of the royal children. He was highly rewarded by the Emperor and appointed court physician, with a generous salary for the rest of his life.

In 1769 he journeyed to Florence, at the request of the Empress, to vaccinate the future Kaiser Leopold II. INGEN-HOUSZ undertook this mission

with great reluctance. The nervous strain of vaccinating so many promi-
nent persons was telling upon him, and he wished to escape further honors
and seek peace. This vaccination also was highly successful, to the great
delight of the Empress. INGEN-HOUSZ lingered some time in Florence to
regain his health and to visit with the physicist, ABBÉ FONTANA. This visit
influenced his later researches on the gas exchange of plants.
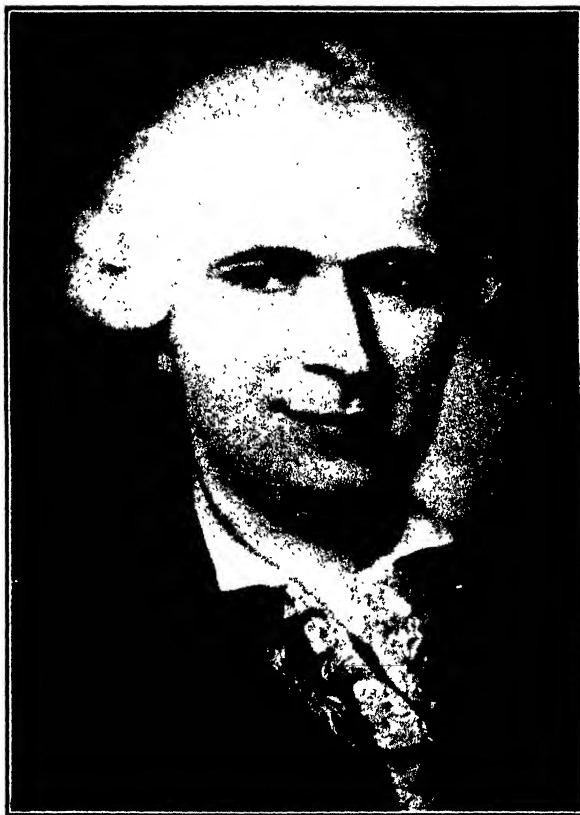
FIG. 1. INGEN-HOUSZ, from a contemporary pastel.

The appointment of INGEN-HOUSZ as court physician at Vienna had a
great influence on his life, for it gave him the means and leisure to carry
on the researches in science, in which he was so interested. After his return
from a second Florentine trip until the end of his life he devoted himself
to research. He desired to travel and visit with other scientists. He vis-
ited Switzerland in 1770, and the next year saw him in Paris, Holland, and
England, then back to Vienna.

In 1775 he married AGATHA JACQUIN, the sister of the botanist, NICOLAS
JACQUIN, professor of botany and chemistry at the University of Vienna.

Three years later found him again in England, where he began his research in plant physiology and physics and wrote his chief work: "Experiments upon Vegetables, discovering their great power of purifying the common air in the sunshine and of injuring it in the shade and at night."

. Before going back to Vienna in 1780, he visited Paris, principally to make the acquaintance of BENJAMIN FRANKLIN, who was interested in a subject on which INGEN-HOUSZ later worked—the heat conduction of metals. He also visited FONTANA in Paris, being especially interested in the eudiometer method, which he found of the greatest use in his work on the gas exchange in plants.

FIG. 2. Bust of INGEN-HOUSZ in the Arcade at the University of Vienna.

INGEN-HOUSZ remained then eight years uninterruptedly in Vienna. To this period belongs his work on carbon dioxide assimilation in plants, in which he was assisted by Drs. MOLITOR, SCHERER, PICHEL, and his nephew, the younger JACQUIN. Most of his chemical and physical researches were carried on during this period in Vienna.

Kaiser JOSEF was very friendly with INGEN-HOUSZ and interested in his researches; he often visited his laboratory and watched him perform experiments. INGEN-HOUSZ's knowledge of electricity and magnetism was not exceeded by that of the famous members of the French Academy, BAILLY, GUILLOTIN, JUSSIEU, LAVOISIER, and LEROY.

In the summer of 1788 INGEN-HOUSZ again went to France. He arrived in Paris on the 14th of July, the day of the taking of the Bastille. This event upset his plans so that he decided to go to America to visit his friend, FRANKLIN. However, news reached him that his only brother had been

killed in an accident, which again changed his plans, and he started for Breda. The journey was very hard for him. He had to travel through Belgium, which had just freed itself from Austria. His position at the Austrian court made him very unpopular, and a number of unpleasant experiences marred his journey. He left Breda after a stay of one week and went to London. Here he learned of the death of FRANKLIN and gave up his American trip.

Depressed by the deaths of his two friends and exhausted by the trials of his journey, his health gave way. He took the advice of his English friends and decided to remain in England until political conditions in Europe warranted his attempting to journey back to Vienna in safety. But, as events turned out, he never again returned to Austria; for during the remaining eleven years of his life, conditions never prevailed in which he felt equal to attempting the journey back—much as he longed to go, as many letters to his wife and nephew indicate.

He remained in London, working or visiting his friend, DIMSDALE, at Herforth, or Lord LANSDOWNE, the statesman and patron of the arts and sciences. At Lord LANSDOWNE's estate at Bowood, his health improved and again he took up his researches on the nutrition of plants. Lord LANSDOWNE fitted up a laboratory for him. Here he wrote his second work: "An Essay on the Food of Plants and the Renovation of Soils." Agriculture of the Counties of Britain 14: 1–20, 1794–1795.

INGEN-HOUSZ died on September 7, 1799, after being in ill health for a considerable time. To the last, he hoped and planned to return to Austria, his adopted land. He was buried with great honors, in England, but the place of burial is not known with certainty. Fig. 1 is reproduced after a pastel made during his life, and fig. 2 shows the bust modeled by F. Seifert, which is now in the Arcade at the University of Vienna.—R. B. HARVEY, and HELEN M. WHITTIER HARVEY, *University of Minnesota.*

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NOTES

Program Committee.—Active preparations are being made for the Cleveland meeting of the American Society of Plant Physiologists, in December 1930. The president of the Society, Dr. S. V. EATON, has appointed the program committee, as follows: Prof. J. H. GOURLEY, of the Ohio Agricultural Experiment Station and Ohio State University, chairman; Dr. JOHN W. SHIVE, of Rutgers University; Dr. W. J. HIMMEL, of the University of Nebraska; and Dr. A. R. DAVIS, of the University of California. The secretary-treasurer is *ex-officio* a member of the committee, also. The geographical distribution of this committee corresponds very well to the regional interests of the Society, and the chairman is located near the meeting place, so that arrangements can be given close personal attention. All of us can help the committee in its tasks by cooperating in any way that seems desirable. When papers are solicited for program purposes, a prompt and generous response would be greatly appreciated. Members may be called upon for other services, and the program committee should be able to command the active support of every member for that meeting.

Committee on Analytical Methods.—A slight change has occurred in the committee on methods of analysis. During the last several years Dr. W. E. TOTTINGHAM, of the Department of Agricultural Chemistry, the University of Wisconsin, has been chairman of this committee. At his request he has been relieved of the chairmanship, and Dr. J. J. WILLAMAN, head of the Department of Agricultural Chemistry of the New York Agricultural Experiment Station, has consented to become the leader of this important committee. Fortunately Dr. TOTTINGHAM remains a member of the committee which he has led so ably during the last several years. His service during this period deserves the thanks of all.

The de Vries and Ingen-Housz Portraits.—The portrait of DE VRIES in the January 1930 number of PLANT PHYSIOLOGY, and of INGEN-HOUSZ in this number, can be purchased from the editor of PLANT PHYSIOLOGY at 12 cents each. There are now six of these portraits of famous plant physiologists available, and the complete set can be obtained for office and laboratory use for 72 cents, postage paid. Postage stamps are accepted for all bills, and are preferred to checks from outside of Chicago, since the banks charge for collection on outside checks. The science of plant physiology has a fascinating and inspiring historical background. Developing students should have an opportunity to know about, and to appreciate the great scientists whose work forms the foundations upon which we are build-

ing today. Those desiring portraits should not wait until the earlier ones have been exhausted.

Stephen Hales Prize Fund.—The plans for enlargement of the STEPHEN HALES Prize Fund have been placed in the hands of a committee, consisting of Dr. R. P. MARSH, of Gettysburg College, and the Secretary-Treasurer. A letter has been sent out to the members, inviting cooperation in this enterprise. Would it not be a very appropriate thing to double the present fund? Every member of the Society has a right to feel proud of the fact that this fund has been established in honor of a great experimentalist, and that the fund will continue to stimulate and reward those who follow worthily in the footsteps of the forefathers of plant physiology. If each one will share in a small way in the privilege of contributing to the fund, the results will be gratifying to everybody.

The Congresses.—Attention is called again to the Student Third Cabin Association which offers on the Holland-America Line attractive accommodations to anyone who plans to attend the international congresses which occur in Europe this summer. If you have not decided upon the manner in which you expect to reach these meetings, it would be profitable to investigate the S. T. C. A. way. An inquiry addressed to Mr. J. S. ROBBINS, passenger office of the Holland-America Line, 21–24 State St., New York, will bring full information about their accommodations, sailing dates, rates, etc.

International Address List.—The second bulletin of the American Society of Plant Physiologists, published in May 1924, was an international address list of plant physiologists. It proved to be a very valuable contribution. Our members will all be pleased to know that a second edition of this bulletin has been planned, to bring up to date this interesting and valuable list. The plant physiologists of the entire civilized world are figuratively "speaking the same language." There is something in the subject which draws us all together in bonds of friendship and fellowship. If the address list helps us all to know one another better, by facilitating exchange of ideas and helpful criticisms, and by promoting the solidarity of the group, it will serve a very useful function. Further announcements may be expected with reference to this bulletin.

Wild Flower Seeds.—If the seeds of western species of flowers are needed for research, it is possible to obtain seeds of many of the native species of California from Lester Rowntree and Co., of Carmel, California. Their recent list contains the names of several hundred different species, but prices are not quoted on many of them. Want lists would undoubtedly receive courteous attention.

Recent Advances in Plant Physiology.—A book reviewing the recent advances in plant physiology has been prepared by E. C. BARTON-WRIGHT, lecturer in King's College, University of London. While it is impossible to summarize all of the literature, the author has done well in his attempt to select the material in such a way as to mirror the advancement of the subject during the last 15 years. The order of presentation is logical, root and soil relations, transpiration and translocation, carbon and nitrogen metabolism, respiration, growth and development. The book will be especially useful to students who need a brief orientation to the recent progress. It is fortunate that some interest is being taken in the need for good works in this field. The price of the book is $3.50, and the publishers are P. Blakiston and Son, Philadelphia.

Hydrogen Ion Concentration in Plant Cells and Tissues.—The second volume of the Protoplasma Monographien from the press of the Gebrüder Borntraeger, of Berlin, is this work on Hydrogen Ion Concentration by Dr. JAMES SMALL, Professor of Botany in Queen's University of Belfast. There are 20 chapters, several appendices, and 25 pages of literature citations. The three sections into which the work is divided are the Introduction, presenting the problems, proteins, enzymes, buffers, sap, protoplast, and wall, and variation in reaction; Methods, including hydrogen electrode, quinhydrone electrode, micro-hydrogen electrodes, comparator indicator methods, capillator indicator, special indicator, range indicator, and buffer determinations; and Results, which gives a general survey of tissue reactions, diurnal and seasonal variation studies, and the data from individual plants, such as the sunflower, broad bean, potato, succulents, etc. The later chapters of the Results section take up the protoplast and pH, cell sap and pH, cell wall and pH, buffers and buffer indexes in plants. Chapter 20 restates the problems. It is a helpful summary in English of the whole field of hydrogen ion concentration. The price of the book bound in cloth is 30 RM. Orders should be sent to the publishers direct, W 35 Schöneberger Ufer 12 a, Berlin.

Laboratory Manual of Plant Physiology.—An attempt to revise the Detmer Praktikum having been made without success, Dr. L. BRAUNER, Privatdozent in the University of Jena, has prepared what is really a new book under the old title, Das kleine pflanzenphysiologische Praktikum. Part I, which is the only part yet received, deals with the chemistry and physics of the materials found in the plant body. Three other parts are projected, one dealing with the physical chemistry of the plant and its environment, another with the chemistry of metabolism, and a final section with structural modifications and movements. Part I contains 125 experiments,

covering the inorganic materials, and 13 groups of organic compounds. These experiments offer many suggestions for a successful course in the chemistry of plant physiology. In brochure form it is obtainable for 5.5 RM, and bound, for 7 RM. The publisher is Gustav Fischer, Jena, Germany. Plant physiologists engaged in laboratory teaching or investigation will find it a very useful guide.

EFFECTS OF INSOLATION AND SOIL CHARACTERISTICS ON TISSUE FLUID REACTION IN WHEAT

W. F. LOEHWING

(WITH ONE FIGURE)

Introduction

In an earlier investigation of certain grain plants grown on humus soils, foliar chlorosis was frequently observed following use of lime to correct soil acidity (31). Tissue fluids of plants from alkalinated humus soils were found to be uniformly low in free acidity, and iron chlorosis was observed in plants in the low acid ranges. The prevalence and increasing severity of chlorosis during continued periods of intense insolation led to the investigation described herein to determine the effect of light on the acidity of sap, and the mobility of iron, in wheat. A number of investigators (9, 13, 14, 23, 53) have shown that the acidity of tissue fluids may decrease in light but there is as yet no wholly reliable evidence that this diminution in acidity during ordinary daylight is sufficient to interfere with the solubility and translocation of iron. In fact, it appears that under optimal conditions of nutrition, light does not always appreciably diminish the acidity of non-succulents 24, 46). The correlation of strong light with chlorosis in plants grown on humus soils low in mineral content, however, suggested that under such conditions the effects of insolation might be sufficiently accentuated to cause injury.

Methods

Two sets of Marquis wheat were grown in a greenhouse at 20° C. One set of plants was grown on a strongly acid humus, low in mineral matter, and another set on a fertile but acid loam. Approximately half of the soil in each set was treated with 2000 parts per million of powdered calcium carbonate. When the plants were six weeks old a thin, white muslin screen was fastened above half of the plants in both the limed and unlimed series in order to reduce the intensity of the illumination. The remaining cul-

tures were left exposed to direct light. Though old and young leaves displayed differences in the degree of acidity, the general trend of hydrion variations under comparable conditions was consistently the same in all parts of the tops. This fact made it feasible to analyze the sap expressed from entire tops without risk of obliterating significant differences among the tissue fluids of variously treated plants.

Entire tops of several plants in each set were cut at consecutive four-hour intervals. These tops were immediately placed in large stoppered vials and frozen at $-30°$ C. Tissues were cut in a Nixtamal mill and the sap was then expressed from thirty gram samples under uniform pressure in a chilled steel press. Duplicate potentiometric determinations by means of the calomel half cell and quinhydrone electrode method (8) were rapidly made at $20°$ C. on 5-cc. aliquots. Three series of hydrion measurements are reported. The first analyses (table I) were started on the morning the muslin screen was placed over a portion of the six week old cultures. The second series of measurements (table II) was made on the sap of eight week old plants following a period of strong insolation during which symptoms of iron chlorosis had become conspicuous in the younger leaves of plants on the limed humus. Moisture content of the fresh tops of eight week old plants varied between eighty-four and eighty-eight per cent. of the fresh weight. The final analyses (table III) show the acidity and moisture content of ten week old chlorotic plants.

Though freezing and maceration of tissues to some extent alter the pH value of expressed sap this treatment was found to give more uniformity in the acidity determinations of aliquots from similar cultures than in sap expressed from unfrozen tissues (35). No attempt was made to eliminate solids suspended in the sap because of negligible differences between centrifuged and uncentrifuged aliquots. The difficulty in keeping samples chilled while centrifuging and the time consumed in the process caused greater pH fluctuations than those due to solids in the highly fluid type of saps obtained.

Determination of acidity in sap expressed by pressure has been criticized by REA and SMALL (44) because it furnishes no very obvious clues as to the processes by which it is brought about. Investigation of expressed sap has not, however, proven entirely fruitless because the degree of acidity has been found to be measurably correlated with illumination (9, 12, 14, 23, 46, 51, 53), the photoperiod (13), temperature (3, 26), age (3, 4), season (1, 22), the axial gradient (11, 28), and nutrient conditions (6, 7, 10, 31, 36). Discovery of such correlations is often the initial step in the determination of their underlying physiological mechanisms. Aided by knowledge of hydrion ranges and buffer indices in certain plants, MARTIN

(32, 33, 34), by a study of expressed sap, has very effectively identified important components of the plant buffer mechanism.

Difficulty in differentiating tints seriously limits the utility of intravital staining methods (27, 39, 50). These methods involve a definitely circumscribed technique in order to secure the penetration of indicators without cell injury (27). SMALL (50) and his collaborators have shown that the cell wall, cytoplasm and vacuole of a cell may differ distinctly in acidity. Consequently, objections have arisen to the customary usage of the expression "cell acidity" (55). Intravital methods of studying the tissue acidity of plants have not yet surpassed the simpler methods employing expressed sap to determine the causes of changes in reaction.

Discussion

All plants used for the initial acidity determinations (table I) during the sixth week of the experiment were in the tillering stage and apparently healthy in appearance. At the time of the second series of acidity determinations during the eighth week (table II) the majority of the plants on loam soil had ceased tillering and were rapidly elongating. Shaded plants of the same age on the humus soil approached the foregoing in size but not in erectness, vigor or depth of color. Humus plants receiving full illumination lagged behind in stature and maturation. Incipient chlorosis appeared among the unshaded plants on the limed humus soil during the eighth week. Some evidences of chlorosis among the shaded plants on this soil developed about ten days later but at no time became as frequent or severe as among the fully illuminated plants.

The data show that young wheat responded quickly to environmental changes. Fluctuations in sap acidity definitely reflected changes in soil reaction and light conditions. The free acidity of expressed tissue fluids of plants harvested at consecutive four-hour intervals disclosed a regular diurnal cycle with an early morning maximum and a late afternoon minimum in all cultures. The daily periodicity in hydrion concentration may be graphically depicted as a V-shaped curve whose left limb represents the fall from morning maximum and whose right limb represents the subsequent nocturnal rise. Similar decreases in sap acidity during periods of daylight have been reported by HAAS (14) and by TRUOG and MEACHAM (53) for corn, by GARNER (13) and his collaborators for soy-beans, by CLEVENGER (9) for cowpeas, and by HURD (23, 25) for wheat. HURD, however, states that normally the daily sap hydrion fluctuations are insignificant compared to those induced by maturation processes.

The above observations suggest the well-known diurnal hydrion periodicity in succulents due to acid photolysis in light (12, 45, 51, 52). Photo-

TABLE I

SAP HYDRION CONCENTRATION (PH) OF SAP EXPRESSED FROM SIX WEEK OLD
WHEAT PLANTS

HOUR CUT	TYPE OF ILLU-MINATION	HUMUS SOIL		LOAM SOIL	
		UNLIMED	LIMED	UNLIMED	LIMED
6 A. M.	Shaded	5.47	6.22	5.67	6.20
	Full	5.47	6.22	5.67	6.20
10 A. M.	Shaded	5.51	6.28	5.68	6.42
	Full	5.57	6.42	5.68	6.42
2 P. M.	Shaded	5.55	6.30	5.69	6.44
	Full	5.62	6.51	5.70	6.46
6 P. M.	Shaded	5.59	6.38	5.70	6.46
	Full	5.69	6.63	5.74	6.51
10 P. M.	Shaded	5.55	6.40	5.69	6.45
	Full	5.64	6.62	5.71	6.51
6 A. M.	Shaded	5.49	6.18	5.64	6.17
	Full	5.50	6.18	5.65	6.22

sensitive carboxylic acids have been reported in certain non-succulents (34, 50) and they may be rather widely distributed in small amounts. It is thus possible that slight reductions in the sap acidity of non-succulents in light may also be ascribable to acid photolysis. This daily acid rhythm may also be caused in part by the assimilation of some component of the buffer system during the normal metabolism of the plant. No definite information is as yet available as to the nature of the variable component of the sap responsible for its daily pH fluctuations. Although SAYRE (47) and SCARTH (48, 49) have shown that rather small variations in the sap acidity of guard cells are peculiarly important in the regulation of stomatal apertures, it is doubtful if the small daily fluctuations in the water content of leaves measurably influence acid periodicity of entire tops (29, 30, 47). Daily acid periodicity does not, however, appear to be as wide-spread nor as accentuated in non-succulents as in cacti.

On account of their obvious importance, studies on the photosynthetic and tropic effects of light on plants rightly hold a preëminent place in physiological research. Much recent work, however, indicates that light is directly important to plants in a number of other ways. Investigation of these less obvious effects of light would probably clarify many plant processes and might incidentally reveal the mechanism of diurnal acid

TABLE II

SAP HYDRION CONCENTRATION (PH) OF SAP EXPRESSED FROM EIGHT WEEK OLD
WHEAT PLANTS

HOUR CUT	HUMUS SOIL				LOAM SOIL	
	UNLIMED		LIMED		UNLIMED	LIMED
	UNSHADED	SHADED	*UNSHADED	SHADED	UNSHADED	UNSHADED
6 A. M.	5.55	5.47	6.12	6.08	5.84	6.03
10 A. M.	5.59	5.50	6.31	6.18	5.89	6.07
2 P. M.	5.67	5.54	6.42	6.36	5.96	6.11
6 P. M.	5.71	5.58	6.51	6.40	5.99	6.24
10 P. M.	5.85	5.61	6.54	6.40	5.97	6.26
2 A. M.	5.57	5.54	6.38	6.20	5.92	6.12
6 A. M.	5.59	5.49	6.22	6.17	5.89	6.05
10 A. M.	5.65	5.52	6.30	6.19	5.93	6.08
2 P. M.	5.72	5.56	6.51	6.35	5.98	6.16
6 P. M.	5.79	5.56	6.67	6.35	6.03	6.31
10 P. M.	5.72	5.54	6.67	6.28	6.01	6.26
6 A. M.	5.50	5.61	6.25	6.21	5.90	6.10
10 A. M.	5.67	5.55	6.38	6.28	5.96	6.17

* Plants of this series showed incipient chlorosis.

periodicity in non-succulent plants. The investigations of WEISMANN (56, 57), NĚMEC and GRACANIN (37), HOAGLAND and DAVIS (21) and WLODEK (58) suggest that light more or less directly influences the intake of mineral nutrients by plants. Certain catalytic but non-photolytic effects of light observed by PRIESTLEY (41) and others (5, 43, 54) profoundly affect plant development. In addition to the above, there are direct effects of light on enzymatic activity (38) and the photocapillary effect described by HERČÍK (15, 16, 17).

It will be noted (fig. 1) that fluctuations occur from day to day in the hour and level of maximal and minimal acidity but the rhythmic rise and fall continued until the end of the experiment in all except the most chlorotic plants. The variation in the time and degree of the afternoon minimal acidity was, however, consistently greater than the variation in the morning maxima. The steepness in slope of the acidity curve appears to be determined primarily by the hour and level of the afternoon minimum acidity.

The fact that reversals in slope of the diurnal acidity gradient are not coincident with the hour of maximal light intensity suggests that the thermal factor in insolation may not be as important as its light effect. Though no attempt was made to measure foliar temperature, spectral

composition, or thermal energy of the light, it is certain that the thermal maxima of the plants were reached much earlier in the afternoons than the maximum pH of the sap. HURD-KARRER (26) has shown that sap hydrion concentration in wheat rises with the temperature. Thermal and light stimuli thus appear to exert opposite effects on the acidity of tissue fluids in wheat. The fact that sap pH increased in light despite the probable concomitant rise in leaf temperature further suggests the predominance of the photo-effect. It remains a matter for further investigation, however, to determine what portion of the diurnal hydrion variation herein reported is ascribable to temperature increments of the tissues and what fraction directly to light.

Though illumination uniformly diminished sap acidity as described above, differences in light intensity and soil alkalinity exercised considerable influence upon the level of acidity attained. Limed cultures in the shaded and unshaded sets maintained a lower degree of acidity and exhibited a greater initial decrease in sap hydrion concentration than the corresponding untreated plants exposed to full light intensity. In fact, the pH values of the sap from the limed cultures exposed to strong insolation for several consecutive days (LL, fig. 1) suggests that the period of darkness is too brief for complete acid recovery. Hence acidity falls progressively to lower levels and shows smaller diurnal fluctuations during prolonged periods of strong illumination. Microchemical analyses of leaves show that the diminished acidity of the sap increasingly interferes with iron mobility and finally induces chlorosis such as became apparent in the eight week old plants on the limed humus.

The onset of chlorosis in young leaves was delayed and was less severe in plants which were shaded following the use of lime. This fact, together with the pH values of these plants, suggests that shading to a considerable extent offsets the iron insufficiency created by lime. The acid recovery in shaded lime cultures (SL, fig. 1) does not, however, reach the original level of acidity found in the sap of plants grown on the untreated humus (LU, fig. 1). Microchemical inspection of chlorotic plants disclosed an abundance of iron in the roots but little or none in young leaves.

Considerable interest attaches to the fact that the most vigorous plants grew on the loam soil and the poorest on the fully illuminated, untreated and limed humus. Treated and untreated plants in this soil, though both low in vigor and retarded in development, represent opposite extremes in sap acidity. Seeds in the untreated humus germinated rapidly and the seedlings to all outward appearances grew normally until the fifth week. The rate of stem elongation then diminished and the leaves remained narrow though increasing in length. During the eighth week the older leaves turned a dull green while their tips became flaccid and turned brown.

The leaves then gradually died back toward the stem. The foregoing symptoms differed from those displayed by plants on the same soil after it had been limed. In the latter instance new leaves became noticeably chlorotic during the eighth week, and the severity of this condition increased with age. Reduced size and delay in maturation marked both the limed and untreated plants on the humus soil. Thus the hyperacidity of the plant on untreated humus on the one hand and the alkalinity of the strongly insolated, limed plants on the other, appeared equally injurious to wheat in its formative stages.
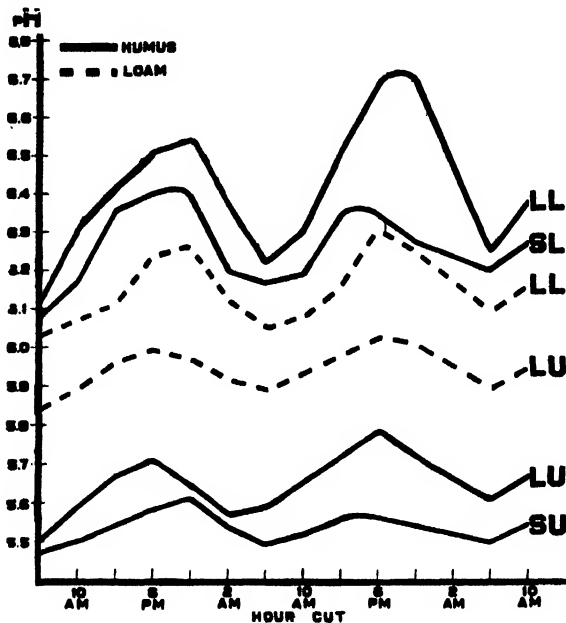
FIG. 1. Diurnal variation of free acidity (pH) in sap expressed from entire tops of eight week old wheat plants. LL, unshaded plants on limed soil; SL, shaded plants on unlimed soil; LU, unshaded plants on unlimed soil; SU, shaded plants on unlimed soil.

During the tenth week, it was found that mature leaves of fully illuminated, chlorotic plants on the limed humus gradually began to lose their turgor and turn brown. Many of the oldest leaves wilted and died. Acidity tests made on these plants during the incipient stages of browning (table III) disclosed that the sap hydrion concentration had risen considerably above that found in the eight week old plants of the same series. Moisture content had also fallen about five per cent. Diurnal acid periodicity could no longer be detected and the hydrion level approximated that of plants growing on the untreated acid humus. This latent increase

TABLE III

SAP HYDRION CONCENTRATION OF FULLY ILLUMINATED, CHLOROTIC PLANTS (TEN
WEEKS OLD) ON LIMED HUMUS

HOUR CUT	pH
6 A. M.	5.57
10 A. M.	5.55
2 P. M.	5.59
6 P. M.	5.59
10 P. M.	5.62
6 A. M.	5.58
10 A. M.	5.55
2 P. M.	5.55
6 P. M.	5.58

in the acidity of initially low-acid chlorotic plants is probably due to the
accumulation of acid catabolic products and to the decrease in tissue
fluids. The entire symptom complex of these plants reflects an incoordina-
tion of metabolic processes which finally culminated in acid toxicity. Thus
the extremes of high and low soil acidity both eventually result in sap
hyperacidity. Though acidity generally increases with rises in tempera-
ture, it appears that opposite extremes in temperature also culminate in
acid injury (26) and it is possible that persistent lack of vigor, irrespective
of its mode of origin, may after a sufficient lapse of time result in high
sap acidity (23, 25, 26).

The untreated loam produced more vigorous plants than the untreated,
low-mineral humus though both soils were strongly acid. In this connec-
tion reference may be made to the work of ÅSLANDER (2) who has shown
that the concentration of nutrients is apt to be more significant than the
pH of the soil or culture medium. He has shown that calciphiles may
develop normally in highly acid media provided the latter contain suffi-
ciently concentrated nutrients. Acid injury occurred only when highly
buffered acid cultures were diluted without change in pH. Even in such
instances plants again developed normally if the hydrion concentration of
the diluted cultures was reduced. ÅSLANDER concludes that many plants
are highly acid tolerant if soils are rich in salts. The work of HOAGLAND
(19)also indicates that increased availability of nutrients may cause an
increase in sap alkalinity. In the present instance the higher mineral
content of the acid loam may in part account for the better growth of
wheat on this soil.

It will be noted (table II) that fluctuations in light intensity and soil
acidity produced smaller changes in sap hydrion concentration of plants

grown on the loam soil. The greater mineral content of the loam probably results in more highly buffered tissue fluids and these in turn show smaller fluctuations in free acidity. Recent work (50) has definitely established the importance of mineral nutrients as buffers in plant tissue fluids. Plants adequately supplied with inorganic salts may be able by the buffer effect of these to maintain a fixed pH level and to protect themselves against acid injury. Under certain conditions plants also appear to supplement the protective effect of their buffer systems. HOAGLAND (19, 20) and his co-workers have shown that absorption of nitrates by plants is increased in acid media. PRIANISCHNIKOW (40) states that this increased absorption of nitrates from acid soils is followed by active excretion of ammonia resulting in neutralization of acidity about the roots.

The data of other investigators suggest that the effect of lime on sap acidity varies with the species and with the soil. CLEVENGER (9) reports that lime fertilizers applied to the same soil increased the sap hydrion concentration in oats and soybeans but not in buckwheat. HAAS (14) and other workers also report increased acidity in some species and diminished acidity in others following liming. Though the direction of the shift in pH appears to vary with the type of plant and the soil, HAAS and CLEVENGER have shown that lime exerts a profound effect on tissue fluid reaction in all instances. Under certain conditions, such as lack of balance among nutrients or mineral insufficiency, it seems that the effect of lime in altering the free acidity of the sap may outweigh its other functions as a nutrient.

The foregoing data show that both soil reaction and light influence sap acidity. When their effects are cumulative the change in metabolism is sufficient to cause a gradual alteration in the appearance of the plants. Neither the effect of light, nor of mineral nutrients, on sap hydrion concentration can be explained on any simple basis. The manifold importance of light as a factor influencing the carbohydrate supply, water content, mineral nutrient intake and sap acidity of plants makes it exceedingly difficult to interpret its effects. We are especially in need of additional information concerning the effects of insolation on mineral nutrition and tissue reaction. Considerable evidence stressing the importance of sap acidity as a morphogenetic factor in plant growth has already accumulated (3, 13, 17, 18, 42) but its accurate interpretation awaits further information concerning the factors controlling the reaction of tissue fluids.

Summary

Tissue fluids from entire wheat tops showed diurnal acid periodicity, free acidity reaching a minimum in the evening and a maximum in the early morning. Sap acidity of wheat plants on acid soils was much greater

than that of plants on the same soils after applications of calcium carbonate to correct acidity. The general level of free acidity was much lower in plants on an untreated, low mineral humus than in those on an acid loam higher in mineral matter. Symptoms of acid toxicity appeared in the tops of eight week old wheat on the untreated acid humus. Following correction of soil acidity with lime, the sap hydrion concentration of plants on the humus was reduced more than in those on the loam. The sap acidity of strongly insolated plants on limed humus soil fell below the level necessary for iron mobility, as shown by chlorosis and absence of iron in the leaves. When chlorosis had persisted for two or more weeks, leaves gradually lost their turgor and acidity rose rapidly as the moisture content diminished.

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LITERATURE CITED

1. ABBOTT, O.　Chemical changes at beginning and ending of rest period in apple and peach. Bot. Gaz. **76**: 167–184. 1923.
2. ÅSLANDER, A.　Concentration of the nutrient medium versus its hydrogen-ion concentration as manifested by plant growth. Svensk Bot. Tidskr. **23**: 96–140. 1929.
3. ASTRUC, A.　Recherches sur l'acidité végétale. Ann. Sci. Nat. Bot. (Series 8) **17**: 1–108. 1903.
4. BARTHOLOMEW, E. T.　Internal decline of lemons: II. Growth rate, water content, and acidity of lemons at different stages of maturity. Amer. Jour. Bot. **10**: 117–126. 1923.
5. BATALIN, A.　Ueber die Wirkung des Lichtes auf die Entwickelung der Blätter. Bot. Ztg. **29**: 669–686. 1871.
6. BAUER, F. C., and HAAS, A. R. C.　The effect of lime, leaching, form of phosphate and nitrogen salt and soil acidity, and the relation of these to the feeding power of the plant. Soil Sci. **13**: 461–480. 1922.
7. BENNETT, J. P., ANDERSSEN, F. G., and MILAD, Y.　Methods of obtaining tracheal sap from woody plants. New Phytol. **26**: 316–323. 1927.
8. CLARK, W. M.　The determination of hydrogen ions. Williams and Wilkins, Baltimore. 1928.
9. CLEVENGER, C. B.　Hydrogen ion concentration of plant juices. Soil Sci. **8**: 217–242. 1919.
10. DUSTMAN, R. B.　Inherent factors related to absorption of mineral elements by plants. Bot. Gaz. **79**: 233–264. 1925.

11. Gustafson, F. G. Hydrogen-ion concentration gradient in plants. Amer. Jour. Bot. **11**: 1–6. 1924.

12. ——————. Diurnal changes in the acidity of *Bryophyllum calycinum*. Jour. Gen. Physiol. **7**: 719–728. 1925.

13. Garner, W. W., Bacon, C. W., and Allard, H. A. Photoperiodism in relation to hydrogen-ion concentration of cell sap and carbohydrate content of the plant. Jour. Agr. Res. **27**: 119–156. 1924.

14. Haas, A. R. C. Studies on the reaction of plant juices. Soil Sci. **9**: 341–369. 1920.

15. Heřčík, F. The photocapillary reaction of plant sap. Biochem. Jour. **21**: 1253–1258. 1927.

16. ——————. Photocapillare Reaktion des Pflanzensaftes. Biol. listy **13**: 108–130. 1927.

17. ——————. Die photoelektrischen Grundlagen der photokapillaren Reaktion. Protoplasma **5**: 400–411. 1929.

18. Herklots, G. A. C. The effects of an artificially controlled hydrion concentration upon wound healing in the potato. New Phytol. **23**: 240–255. 1924.

19. Hoagland, D. R. Relation of the concentration and reaction of the nutrient medium to the growth and absorption of the plant. Jour. Agr. Res. **18**: 73–117. 1919.

20. ——————, and Davis, A. R. The composition of the cell sap of the plant in relation to the absorption of ions. Jour. Gen. Physiol. **5**: 629–646. 1923.

21. ——————, ——————. Further experiments on the absorption of ions by plants, including observations on the effect of light. Jour. Gen. Physiol. **6**: 47–62. 1923.

22. Hooker, H. D. Seasonal changes in the chemical composition of apple spurs. Missouri Agr. Exp. Sta. Res. Bull. 40. 1920.

23. Hurd, A. M. Hydrogen-ion concentration and varietal resistance of wheat to stem-rust and other diseases. Jour. Agr. Res. **23**: 373–386. 1923.

24. ——————. Acidity of corn and its relation to vegetative vigor. Jour. Agr. Res. **25**: 457–469. 1923.

25. ——————. The course of acidity changes during the growth period of wheat with special reference to stem-rust resistance. Jour. Agr. Res. **27**: 725–735. 1924.

26. Hurd-Karrer, A. M. Relation of leaf acidity to vigor in wheat grown at different temperatures. Jour. Agr. Res. **39**: 341–350. 1929.

27. Ingold, C. T., and Small, J. The hydrion concentration of plant tissues. IX. Improved technique for the R. I. M. Protoplasma **3**: 458–468. 1928.

28. KAPPEN, H. Untersuchungen an Wurzelsäften. Landw. Versuchs.-Sta. **91**: 1–40. 1918.

29. KNIGHT, R. C. Further observations on the transpiration, stomata, leaf water-content and wilting of plants. Ann. Bot. **36**: 361–383. 1922.

30. LIVINGSTON, B. E., and BROWN, W. H. Relation of the daily march of transpiration to variations of the water content in foliage leaves. Bot. Gaz. **53**: 309–330. 1912.

31. LOEHWING, W. F. Calcium, potassium, and iron balance in certain crop plants in relation to their metabolism. Plant Physiol. **3**: 261–275. 1928.

32. MARTIN, S. H. The hydrion concentration of plant tissues. IV. The buffer of sunflower hypocotyl. Protoplasma **1**: 522–536. 1927.

33. ——————. The hydrion concentration of plant tissues. VII. The buffers of sunflower stem and root. Protoplasma **3**: 273–281. 1928.

34. ——————. The hydrion concentration of plant tissues. VIII. The buffers of bean stem and root. Protoplasma **3**: 282–301. 1928.

35. MEYER, B. S. Some critical comments on the methods employed in the expression of leaf saps. Plant Physiol. **4**: 103–112. 1929.

36. MIYAKE, K., and ADACHI, M. Influence of manurial ingredients upon the hydrogen ion concentration of the juice of rice plant. Jour. Biochem. **5**: 321–326. 1925.

37. NĚMEC, A., and GRACANIN, M. Influence de la lumière sur l'absorption de l'acide phosphorique et du potassium par les plantes. Compt. Rend. Acad. Sci. (Paris) **182**: 806–808. 1926.

38. PINCUSSEN, L. Fermente und Licht I. Diastase I. Biochem. Ztschr. **134**: 459–469. 1923.

39. PFEIFFER, H. Über die Wasserstoffionenkonzentration (H·) als Determinationsfaktor physiologischer Gewebegeschehen in der sekundären Rinde der Pflanzen. New Phytol. **24**: 65–98. 1925.

40. PRIANISCHNIKOW, D. Über die Ausscheidung von Ammoniak durch die Pflanzenwurzeln bei Säurevergiftung. Biochem. Ztschr. **193**: 211–215. 1928.

41. PRIESTLEY, J. H. Light and growth. I. The effect of brief light exposure upon etiolated plants. New Phytol. **24**: 271–283. 1925.

42. ——————. The meristematic tissues of the plant. Biol. Rev. **3**: 1–20. 1928.

43. ——————, and EWING, J. Physiological studies in plant anatomy. VI. Etiolation. New Phytol. **24**: 30–44. 1923.

44. REA, M. W., and SMALL, J. The hydrion concentration of plant tissues. II. Flowering and other stems. Protoplasma **1**: 334–344. 1927.

45. RICHARDS, H. M. Acidity and gas interchange in cacti. Carnegie Inst. Publication no. 209. Washington, D. C. 1915.

46. ROHDE, K. Untersuchungen über den Einfluss der freien H-ionen im Innern lebender Zellen auf den Vorgang der vitalen Färbung. Pflügers Archiv. **168**: 411–433. 1917.

47. SAYRE, J. D. Physiology of stomata of *Rumex patientia*. Ohio Jour. Sci. **26**: 233–266. 1926.

48. SCARTH, G. W. The influence of H-ion concentration on the turgor and movement of plant cells with special reference to stomatal behavior. Proc. Intern. Congr. Plant Sci. **2**: 1151–1162. 1929.

49. ————. Stomatal movement: its regulation and regulatory rôle. A review. Protoplasma **2**: 498–511. 1927.

50. SMALL, JAS. Hydrogen-ion concentration in plant cells and tissues. Borntraeger Bros. Berlin. 1929.

51. SPOEHR, H. A. Photochemische Vorgänge bei der diurnalen Entsäuerung der Succulenten. Biochem. Ztschr. **57**: 95–111. 1913.

52. ————. Photolysis of plant acids. Carnegie Institution Yearbook **12**: 82–83. 1913.

53. TRUOG, E., and MEACHAM, M. R. Soil acidity: II. Its relation to the acidity of the plant juice. Soil Sci. **7**: 469–74. 1919.

54. TRUMPF, C. Ueber den Einfluss intermittierender Belichtung auf das Etiolement der Pflanzen. Bot. Arch. **5**: 381–410. 1924.

55. ULEHLA, U. Die Regulation der Wasserstoff-ionenkonzentration durch Sukkulenten-Gewebe. Protoplasma **3**: 469–506. 1928.

56. WEISSMAN, H. Über den Einfluss des Lichtes auf die Nahrstoffaufnahme der Pflanzen in Jugendstadium. Ztschr. Pflanzenernähr. und Düngung **4B**: 153–155. 1925.

57. ————. Einfluss des Lichtes auf Wachstum und Nahrstoffaufnahme beim Hafer. Landw. Jahrb. **35**: 183–190. 1919.

58. WLODEK, J. Content of ash constituents and nitrogen in leaves of *Avena sativa*, *Trifolium pratense* and *Phaseolus vulgaris* collected at various times of the day. [Translated title]. Bull. Internat. Acad. Polonaise Sci. et Lett. B. Sci. Nat. 65–78. 1923. Review in Bot. Abst. **15**: 168. (no. 1046). 1926.

TITRATION CURVES OF ETIOLATED AND OF GREEN WHEAT SEEDLINGS REPRODUCED WITH BUFFER MIXTURES

ANNIE MAY HURD-KARRER

(WITH SIX FIGURES)

Introduction

The titration curve for the expressed juice of etiolated wheat seedlings has a pronounced inflection above pH 8.0 (fig. 4), which does not occur in the curve for fully green seedlings (fig. 5). An intermediate type of curve, linear between pH 8.5 and 11.0, is characteristic of very young seedlings just after their emergence from the ground, when, from the standpoint of chemical composition, they are partially etiolated (fig. 6).

The characteristic inflection in the etiolated-seedling curve seemed due to some buffer present in maximum concentration in the absence of chlorophyll, and the change in the form of the curve during the period of seedling development appeared to be caused by a progressive diminution in the amount of this buffer as assimilation processes became established.

The object of the present investigation was to identify this buffer, and to determine whether the titration curves of etiolated and of green wheat seedlings could be reproduced by varying its concentration in chemical mixtures containing it in combination with other buffer compounds known to occur in plant juices.

Material

The etiolated seedlings from which juice was obtained for the representative titration curve in fig. 4 were of the variety White Odessa, grown in the darkness in pure white sand without nutrients at a temperature of 18° C. The plants were eight days old and about 8 cm. high at the time of cutting, with the first leaf just emerging from the coleoptile.

The green seedlings, from which the juice was obtained for the representative titration curve in fig. 5, were of the same variety, grown in soil under normal greenhouse conditions, at approximately the same temperature. The plants were cut eight days after their emergence from the soil. They were 8–10 cm. tall, with one fully expanded leaf.

The characteristic inflection in the etiolated-seedling curve was the same for seedlings grown in soil, pure sand, and on saturated blotting paper without nutrients. The curve for fully green seedlings never showed this inflection whether the plants were germinated in soil or in pure sand without nutrients.

The form of the green-seedling curve varies with age,—from the type suggesting partial etiolation, characteristic of emergent seedlings, to that

obtained a week or two later when the products of assimilation reach an equilibrium concentration and the form of the curve becomes that characteristic of the plant for the remainder of the growth period (26). The curve in fig. 5 was chosen as representative of this final stage, although some variation, apparently due largely to the decreasing water content of the plants, occurs in later stages (26).

Age was not a factor in the shape of the etiolated-seedling curve, which remained essentially unchanged throughout the limited period in which the plants could grow in darkness. However, seedlings younger than five days were not investigated in this connection.

While varietal differences and environmental factors such as temperature and water supply were found to affect the steepness of the titration curve, they did not change essentially the characteristic form associated with etiolation, nor that associated with normal assimilation, as long as growth was normally vigorous. If, however, the plants were not healthy, their condition was reflected in the titration curve. In the case of etiolated seedlings having weak, scraggly growth and poorly developed root systems, the characteristic inflection above pH 8.0 was less pronounced. In the case of green plants, lack of vigor was associated with greatly increased buffer capacity.

The titration values for the juice of plants of the same variety, in the same stage of development, grown under the same environmental conditions, cut at the same time, and handled identically, were remarkably constant. Each of the 45 E. M. F. measurements constituting a complete titration have frequently been reproduced with duplicate samples of juice to within a few hundredths of a pH unit. This reproducibility was particularly marked in the case of etiolated seedlings, for which growth conditions were more constant than they were for plants grown in light.

Methods

To obtain the juice samples, the plants, exclusive of the roots, were ground in a food chopper, and the juice expressed from the pulp by squeezing it through cheesecloth. Ten-cc. samples were titrated electrometrically at a temperature of 25° C. with twentieth normal acid and alkali. The method and equipment are described in an earlier paper (23).

The characteristic slopes and inflections of the plotted titration curves suggested the buffers to be used in the mixtures with which the curves were imitated. To determine their proper concentration, different dilutions of stock solutions of these buffers were titrated, and the concentration of each buffer in the final mixture made equal to its concentration in that solution whose titration curve most nearly coincided with the corresponding section of the juice curve, i.e., the section over which the solute was

apparently the principal buffer. Adjustment of the initial reaction of each mixture to that of the juice, approximately pH 6.00, was brought about by the addition of a small amount of N/1 NaOH. Vertical displacement of the entire curve could be corrected by varying the amount of water in the mixture.

Results

The characteristic inflection of the titration curve for the etiolated-seedling juice (fig. 4) suggested the presence of an amino acid or a related compound. The location of the point of inflection near pH 8.9 indicated that the compound was probably asparagin, which has a dissociation exponent near this point ($pk_a = 8.87$ (7)). Asparagin has been reported to be more abundant in etiolated seedlings than in corresponding seedlings grown in light, owing to its utilization in protein synthesis in assimilating plants (37). WAŚNIEWSKI (50) has shown this relation in the case of wheat seedlings.

To determine whether asparagin was present in the juice of the etiolated wheat seedlings of the present investigation, several samples were evaporated to a thin syrup (after the heat-coagulable material had been removed by filtration) and allowed to stand for some time in a refrigerator, together with similarly prepared samples of green-seedling juice. Many asparagin crystals separated out in the etiolated-seedling preparations, whereas only potassium nitrate crystals appeared in the green-juice samples.[1] Their identity was confirmed by Mr. G. L. KEENAN, Microanalyst of the Food, Drug and Insecticide Administration, by means of the optical immersion method. It was concluded that the distinguishing inflection of the etiolated-seedling curve above pH 8.0 (fig. 4) was due to the asparagin in the juice.

The most important buffer from the standpoint of the physiology of the plant would be one which reacts with acid and alkali at and near the hydrogen-ion concentration of the tissues. The pH values obtained (23, 24, 27) for the juice of healthy wheat plants in the vegetative stage lie within the buffer range of the inorganic phosphates (5, 11). LE CLERC (31) and others have found phosphates to be abundant in wheat seedlings. They have been reported to be important constituents of the buffer systems of a number of plant tissues (1, 12, 28, 33, 34, 35). So it seemed probable that they are of similar importance in wheat.

Accordingly mixtures of asparagin and potassium phosphate were titrated in the first attempts to reproduce the titration curve of the etiolated-

[1] The absence of asparagin crystals in the green-seedling juice can not be taken to mean that asparagin was entirely absent, since crystallization of the relatively small amount undoubtedly there, might have been inhibited by interfering substances which were not present in the etiolated-seedling juice.

seedling juice. In order to minimize final adjustments of the reaction of the mixtures, a stock solution of potassium phosphate was made to have a pH value near that of the juice by adding seven parts of a tenth normal solution of the monobasic phosphate to one part of a similar solution of the dibasic phosphate. It was found after a number of preliminary experiments that the titration values for 10 cc. of a mixture consisting of three parts of this phosphate solution, 13 parts of a tenth normal solution of asparagin, three parts of water, and one drop (0.033 cc.) of N/1 NaOH to adjust the reaction, were very similar to the titration values of the juice between pH 6.0 and pH 9.5. The mixture was lacking in buffer capacity beyond these limits, however.

LEUTHARDT's data (**32**, p. 35) showing that glucose and sucrose react with alkali above pH 9.0 suggested that the addition of sugar to the mixture might give it the buffer capacity of the juice over the extreme alkaline range. After a number of preliminary trials with different concentrations, it was found that a 5 per cent. solution of glucose (1 gram to each 18 cc. of the phosphate-asparagin mixture) was required for the degree of buffer action characterizing the juice above pH 9.5. This concentration is three times that of the total sugar actually found in the juice.[2] So the sugar in the mixture must be regarded as representative of all the soluble carbohydrates of the juice and possibly of other constituents which react with alkali above pH 9.5.

The alkali-titration curve of the etiolated-seedling juice was now reproduced with phosphate, asparagin, and glucose (fig. 1, d). The buffering contributed by the potassium phosphate and by the asparagin is shown in fig. 1 (a and b) by titrations of solutions containing them in the same concentration in which they occurred in the mixture. Their combined buffer capacity is shown also with (fig. 1, d) and without (fig. 1, c) that of glucose. The buffering due to glucose, beginning near pH 9.5, annulled to just the right degree the increase in steepness of the curve above pH 10.0 due to the disappearance of buffering by asparagin.

The mixture still lacked much of the buffer capacity of the juice over the range of the acid titration (fig. 3). Organic acids in the presence of their salts have been shown to be effective buffers in plant tissues over acid reaction ranges (**20, 28, 32, 35**). The identity of these constituents in the wheat plant has not been determined. So in order to determine whether any of the most commonly occurring acids would make possible the dupli-

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[2] In some analyses made by Dr. ALLAN D. DICKSON in this laboratory, 10 cc. of juice from etiolated seedlings were found to contain 0.18 gm. total sugar. The same quantity of juice from green seedlings contained 0.14 gm. The two analyses are not comparable, however, because the temperatures in the greenhouse when the green seedlings were grown were too high for normal growth.
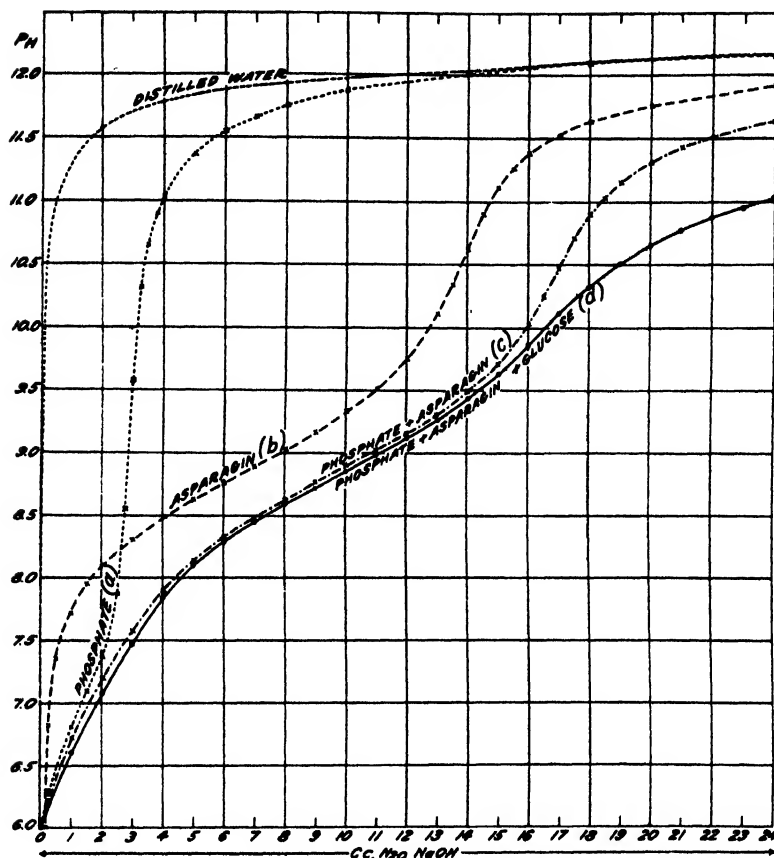
FIG. 1. Graph illustrating the synthesis of the alkali-titration curve of etiolated-seedling juice. The curves of 0.016 M potassium phosphate (a) and of 0.069 M asparagin (b), alone and in combination with each other (c), and with 5 per cent. glucose (d) show the contribution of each buffer to the form of the juice curve. The concentration of the solute in each case was approximately equal to its concentration in the whole mixture.

cation of the acid-titration curve of wheat juice, solutions containing malic, oxalic, citric, tartaric, and succinic acids were titrated for comparison of their curves with that of the juice. At pH 6.0, the approximate reaction of wheat juice, the organic acids are largely, or, in the case of some of them, entirely in the form of salts. So in order that their titrations should be comparable with those of wheat juice, the solutions of the different acids (M/10) were each brought to pH 6.0 by the addition of alkali, adjusted to equal volumes with distilled water, and then titrated similarly with acid. The addition of the necessary alkali and the subsequent volume adjustment with water reduced the original strength of each solution to 0.0645 M.
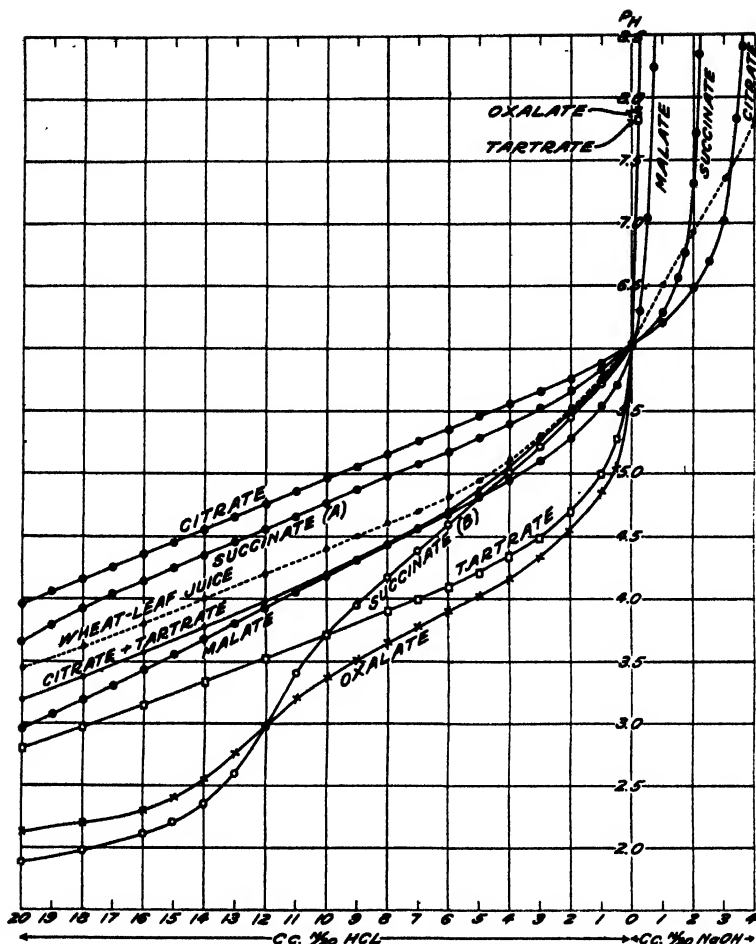
FIG. 2. Graph showing characteristic inflections and buffer zones of plant acids. M/10 solutions of each were first brought to pH 6.0, a reaction typical of wheat juice, by the addition of alkali, then titrated with acid and with alkali, respectively. A titration curve for juice of leaves of 18-weeks-old wheat plants is included for comparison. The concentration of each organic acid-salt mixture was 0.0645 M, excepting a 1:1 dilution (B) of the succinic-acid solution (A), included to show the inflection below pH 4.0.

The titration curves of these solutions were all different, each having characteristic inflections or characteristic zones of buffer action (fig. 2). The malate solution was the only one whose buffer zone was similar to that of wheat juice below pH 6.0. The curves for the oxalate and succinate[3]

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[3] A 1:1 dilution (B) of the original succinate (A) is included in fig. 2 in order to show the inflection below pH 4.0. The corresponding dilutions of the malate, citrate, and tartrate solutions are not included because, owing to the distribution of their dissociation constants, their slopes are practically linear over the entire buffer zone under consideration.
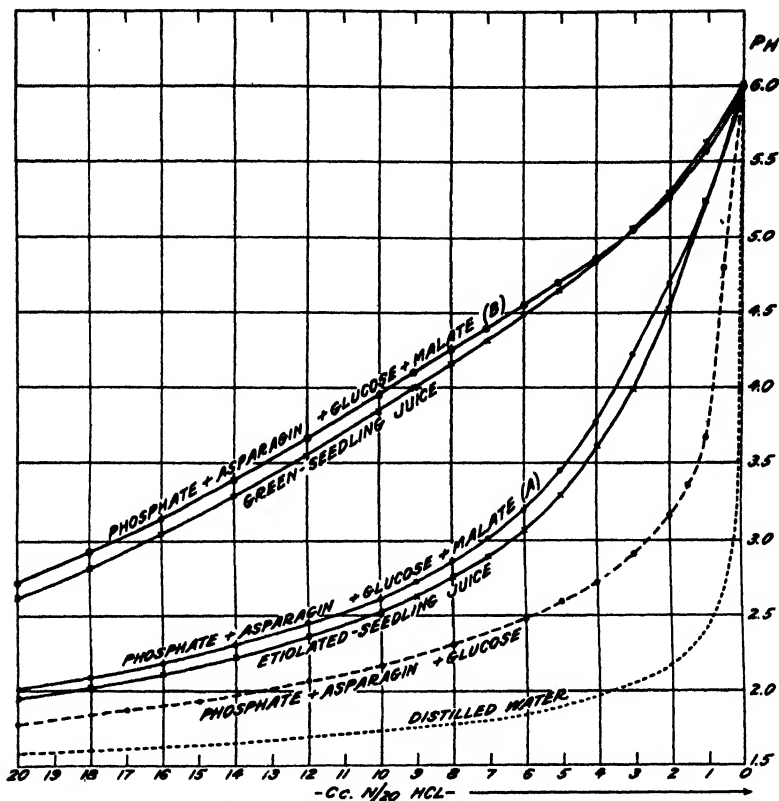
FIG. 3. Graph showing the buffering of the phosphate-asparagin-glucose mixture of fig. 1 against acid, and the effect of adding sodium malate. The concentration of malate in the solution A, imitating etiolated-seedling juice, was 0.013 M; that in the solution B, imitating green-seedling juice, was 0.045 M.

solutions had infections below pH 3.5 and 4.0, respectively, which were never present in the curve for the juice of wheat plants of any age or condition. The citrate solution had too much buffer capacity between pH 5.0 and 6.0 as compared to that below pH 5.0, and the tartrate solution had too little.

Figure 2 shows that although the curve for malates closely resembled that of wheat juice, a combination of citrate and tartrate, in the ratio of two parts of the citrate to three parts of the tartrate solution, gave a curve even more similar to that of the juice. A combination of malate, citrate, and tartrate could be made that would give similar values. Sodium malate alone, however, was chosen for inclusion in the buffer mixture.

A relatively concentrated solution was made by adding enough N/1 NaOH (19.75 cc.) to 10 cc. M/1 malic acid in the electrode vessel to bring the reaction to approximately that of the juice (near pH 6.0). By sub-
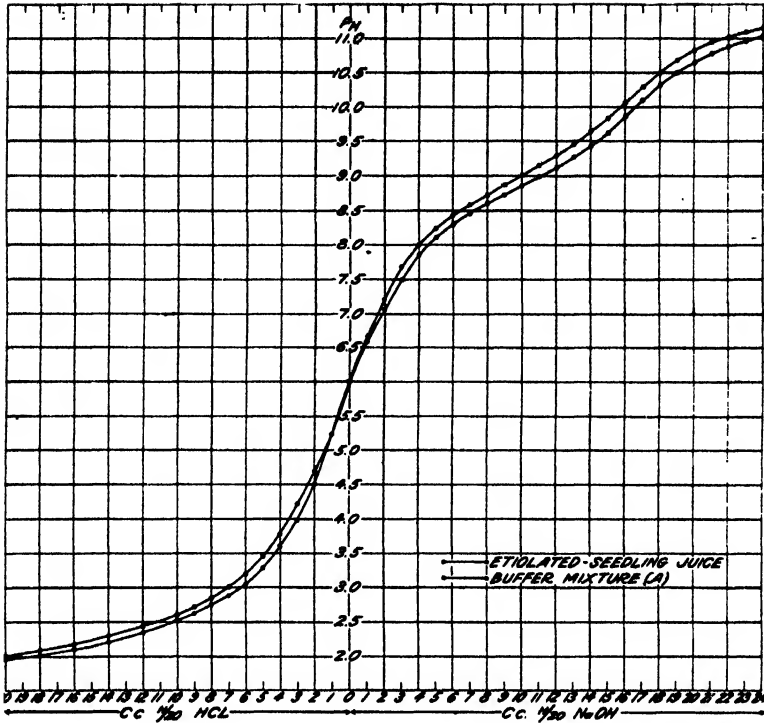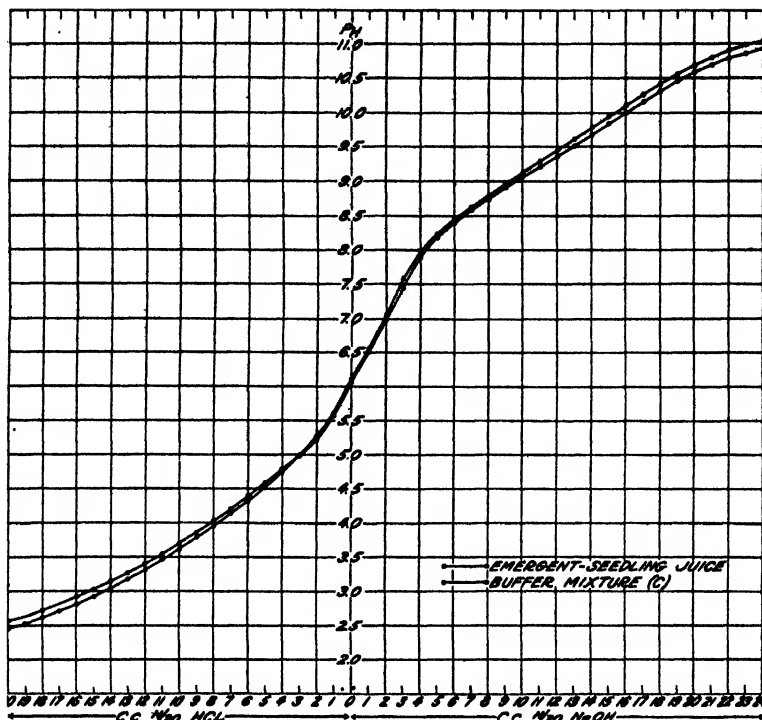
FIG. 4.   Titration curve of juice of etiolated wheat seedlings, and of the buffer mixture
A containing potassium phosphate, asparagin, sodium malate, and glucose.

stituting 1.5 cc. of this approximately M/3 solution for a corresponding
amount of water in each 38 cc. of the phosphate-asparagin-glucose mixture,
with which the alkali-titration curve of the etiolated seedlings had been
reproduced, the mixture was given the desired buffering below pH 6.0
without changing appreciably its alkali-titration values.

In fig. 3 is shown the effect of this addition of malate on the acid titra-
tion values of the phosphate-asparagin-glucose mixture whose alkali-titra-
tion curve is given in fig. 1 (d).   Asparagin has very little buffering power
against acid, but the combined buffering of the asparagin and the phosphate
in the mixture was enough to compensate for the slight deficiency in that
of the malate alone between pH 5.5 and 6.0 and below pH 3.5.

The titration curve of the etiolated-seedling juice was now duplicated
over its entire range with a mixture of phosphate, asparagin, glucose, and
sodium malate (fig. 4).   The exact composition of the mixture (A) is
given in table I on p. 317.

The slight vertical displacement of the synthetic curve as compared to
the juice curve—upward over the range of the acid titration and down-
ward over that of the alkali titration,—is due to a difference in dilution.
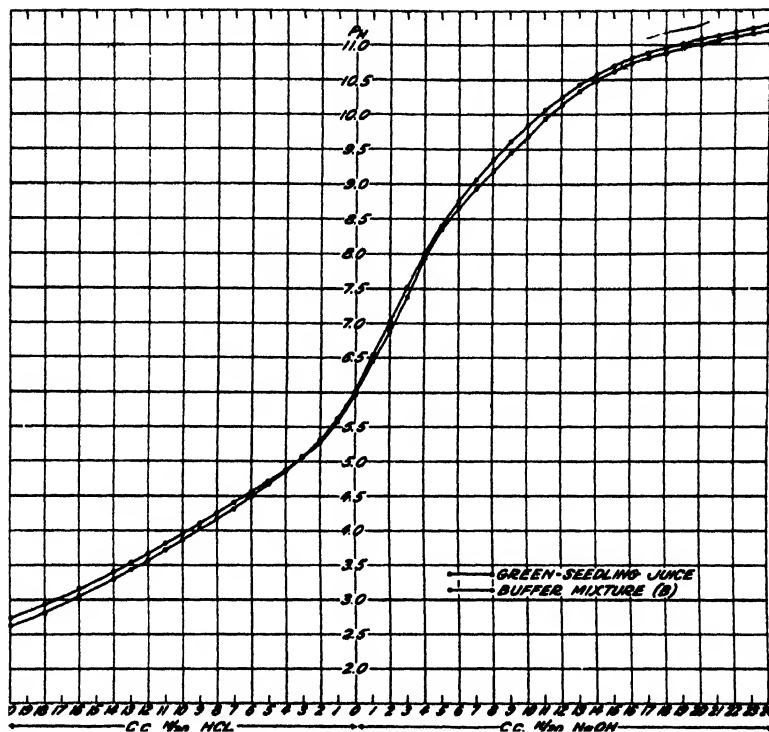
FIG. 5. Titration curve of juice of green wheat seedlings, and of the buffer mixture B containing potassium phosphate, asparagin, leucin, sodium malate, and glucose.

The constituent buffers were all slightly more concentrated in the mixture than they were in this particular sample of juice. The difference was of no greater magnitude, however, than occurs naturally between curves for different samples of juice, so the slight discrepancy was not corrected. In accordance with this difference, all subsequent mixtures were made to have a uniformly higher buffer capacity than the corresponding juices.

The titration curve for green seedlings differs from that of etiolated seedlings in not having the inflection above pH 8.0 and in having considerably increased buffer capacity over the acid range below pH 6.0 (fig. 5). These differences suggested that in order to reproduce it, the quantity of asparagin used in duplicating the etiolated-seedling curve should be decreased, and that of the sodium malate increased.

It was found that the acid-titration values of the green-seedling juice could be reproduced simply by increasing the concentration of sodium malate in the buffer mixture (fig. 3). The concentration of malate in the etiolated-seedling mixture, A, was but 0.013 M, as compared to 0.045 M in the green-seedling mixture, B. So the greater buffering of the juice of

FIG. 6. Titration curve of juice of emergent wheat seedlings and of the buffer mixture
    C containing potassium phosphate, asparagin, leucin, sodium malate and glucose.

green seedlings against acid as compared with that of etiolated seedlings is
apparently due largely to a higher concentration of organic-acid salts.

The difference between the alkali-titration curves seemed due in large
measure to a lower concentration of asparagin in the green seedlings as
compared to that in plants grown in darkness. However, the green-
seedling curve could not be exactly reproduced simply by reducing the
asparagin in the mixture, because the asparagin inflection persisted after
this substance had been reduced to the minimum requisite for the buffer
capacity of the juice between pH 7.5 and 9.5. The problem was then to
add some substance to the mixture which would annul the tendency of the
curve to increase in steepness above pH 9.5, over the region where the
alkali-combining power of asparagin decreases rapidly. Doubling the
amount of sugar accomplished this satisfactorily, but there was already
more sugar in the mixture than was found in the juice. So leucin, which
reacts with alkali at highly alkaline reactions (pk$_a$ = 9.75 according to
BJERRUM (7)), was used instead. The desired effect was obtained by add-
ing M/10 leucin to the mixture in an amount equal to about half that of the
minimum amount of M/10 asparagin required for the buffer capacity of

TABLE I

COMPOSITION OF BUFFER MIXTURES HAVING TITRATION VALUES SIMILAR TO THOSE OF THE
JUICE OF ETIOLATED WHEAT SEEDLINGS (A), FULLY GREEN SEEDLINGS (B),
AND GREEN SEEDLINGS JUST AFTER EMERGENCE FROM THE GROUND (C)

BUFFER	APPROXIMATE BUFFER RANGE pH	COMPOSITION OF BUFFER MIXTURE		
		A "ETIOLATED"	B "GREEN"	C "EMERGENT"
M/3 Sodium malate	2.0– 6.0	1.50 cc.	4.75 cc.	3.80 cc.
M/10 Phosphate	5.5– 8.0	6.0 cc.	6.0 cc.	6.0 cc.
M/10 Asparagin	7.5–10.0	26.0 cc.	9.0 cc.	15.0 cc.
M/10 Leucin	8.0–11.0	——	4.0 cc.	8.0 cc.
Glucose	above 9.5	2 gm.	2.2 gm.	2.2 gm.
Water	——	2.5 cc.	9.0 cc.	——
N/1 NaOH	——	0.033 cc.	0.033 cc.	0.066 cc.

the juice between pH 7.5 and 8.5—over which region neither potassium phosphate nor leucin had much buffer capacity.

The best reproduction of the green-seedling curve obtained is shown in fig. 5, and the exact composition of the buffer mixture (B) is given in table I. The imitation is not perfect, but it is as close an approximation as could be expected with so simple a mixture, lacking as it does a number of the constituents of the wheat plant which react with alkali and acid, such as cholin, betain, and such amino acids as histidin and arginin (13, 43, 51). However, these compounds may not be present in the juice in sufficient amounts to affect appreciably the form of the titration curve.

Table I gives also the composition of a mixture (C) with which the curve typical of normal seedlings just emerging from the ground was reproduced (fig. 6). This mixture contained less asparagin and more sodium malate than did the etiolated-seedling mixture, A, but more asparagin and less sodium malate than did the green-seedling mixture, B. As previously noted (26), the curve for emergent seedlings evidently represents a stage of development when the concentration of the principal buffers is intermediate between that of etiolated and of fully green seedlings.

The titration values of the three buffer mixtures, A, B, and C, are given in table II, together with the values for the corresponding juice titrations. The mixtures show in each case slightly higher buffering over both the acid and alkaline ranges than do the corresponding juice samples. Since the difference is about the same over both ranges,—the synthetic alkali curves falling below and the synthetic acid curves above the juice curves,— the titration values could be brought in still closer agreement by diluting the mixtures with a little water. However, as stated previously,

there seemed no object in exactly superimposing the curves inasmuch as different samples of juice differ even more widely in this respect.

TABLE II

Titration values of buffer mixtures A, B, and C in comparison with corresponding values for etiolated, green, and emergent wheat seedlings

Cc. alkali and acid added to 10 cc.	Etiolated seedling juice	Buffer mixture A	Green seedling juice	Buffer mixture B	Emergent seedling juice	Buffer mixture C
N/20 NaOH	pH	pH	pH	pH	pH	pH
0	6.03	5.99	6.00	5.98	6.09	6.06
1	6.65	6.59	6.53	6.44	6.55	6.51
2	7.20	7.04	7.03	6.85	7.06	6.97
3	7.68	7.48	7.52	7.35	7.58	7.45
4	7.99	7.84	8.00	7.96	7.97	7.89
5	8.23	8.10	8.40	8.37	8.23	8.20
6	8.42	8.29	8.77	8.68	8.44	8.41
7	8.58	8.45	9.08	8.94	8.63	8.59
8	8.72	8.59	9.35	9.20	8.79	8.76
9	8.87	8.72	9.62	9.44	8.96	8.92
10	9.01	8.86	9.86	9.69	9.13	9.07
11	9.15	8.99	10.08	9.94	9.29	9.21
12	9.30	9.12	10.27	10.16	9.46	9.36
13	9.46	9.27	10.45	10.35	9.61	9.51
14	9.64	9.43	10.59	10.51	9.78	9.68
15	9.84	9.62	10.72	10.64	9.95	9.83
16	10.07	9.85	10.82	10.74	10.11	9.99
17	10.29	10.11	10.91	10.83	10.27	10.16
18	10.50	10.32	10.98	10.90	10.42	10.32
19	10.69	10.51	11.04	10.97	10.56	10.47
20	10.83	10.66	11.09	11.03	10.68	10.59
21	10.94	10.78	11.15	11.08	10.80	10.69
22	11.02	10.88	11.20	11.13	10.90	10.79
23	11.10	10.96	11.25	11.17	10.98	10.86
24	11.16	11.03	11.31	11.21	11.06	10.94
N/20 HCL						
0	5.97	5.99	6.00	5.98	6.10	6.06
1	5.23	5.23	5.63	5.58	5.60	5.58
2	4.50	4.69	5.31	5.27	5.26	5.22
3	3.97	4.22	5.05	5.05	4.98	4.97
4	3.59	3.78	4.84	4.87	4.73	4.77
5	3.28	3.45	4.66	4.71	4.52	4.58
6	3.06	3.20	4.49	4.57	4.32	4.39
7	2.88	3.00	4.31	4.41	4.14	4.21
8	2.75	2.84	4.17	4.26	3.96	4.03
9	2.62	2.72	4.04	4.11	3.79	3.86
10	2.52	2.59	3.86	3.96	3.63	3.68
11	2.44		3.72	3.82	3.47	3.54
12	2.35	2.44	3.57	3.67	3.32	3.39
13	2.28		3.43	3.53	3.18	3.26
14	2.21	2.29	3.29	3.40	3.05	3.13
15	2.16		3.17		2.93	3.02
16	2.10	2.17	3.04	3.15	2.81	2.92
17	2.06		2.92		2.72	
18	2.02	2.08	2.81	2.93	2.62	2.72
19	1.97		2.70		2.53	
20	1.94	2.00	2.62	2.73	2.45	2.57

The molar concentrations of each constituent in the three mixtures are given in table III. If the assumption be made that these constituents represent the only buffers having any appreciable effect on the titration values over their respective buffer zones, their concentrations in the mixtures are approximately equal to their actual concentrations in the different juices.

TABLE III

The molar concentrations of the principal buffers in the mixtures used to reproduce the titration curves of wheat-seedling juices

BUFFER	CONDITION OF SEEDLINGS		
	ETIOLATED	PARTIALLY ETIOLATED (EMERGING FROM SOIL)	FULLY GREEN
Asparagin	0.069	0.043	0.026
Organic-acid salt represented by sodium malate	0.013	0.036	0.045ᵃ
Phosphate	0.016	0.017	0.017ᵇ

ᵃ Fig. 3 shows that the concentration of sodium malate required to reproduce the slope of the curve of old wheat plants over the organic-acid buffer range was still greater, i.e., over 0.065 M.

ᵇ Martin (35) reports molar concentrations of phosphate of this order of magnitude in bean juice.

It may be concluded that the green-seedling juice contained about one-third as much asparagin as did that of etiolated seedlings and about three times as much of an organic-acid salt. These differences are consistent with the effects of etiolation on chemical composition as discussed in a following section.

Boiling and subsequently filtering either the etiolated- or the green-seedling juice had no appreciable effect on the distinguishing characteristics of the titration curve. So heat-coagulable proteins play little or no part in the buffer system of wheat, as is true of most other plants, apparently (28, 33, 35, 53). However, an exception appears in the case of potato juice, in which Cohn, Gross, and Johnson (12) found that tuberin has considerable buffer capacity over the range ''acid to pH 4.5 and alkaline to pH 8.5.''⁴

⁴ In a preceding paper by the present writer (26) this statement was referred to as indicating buffer action between these limits, i.e., over the range of the acid and alkali titrations from the pH value of the juice (6.8) to pH 4.5 and 8.5, respectively. From data given in another paper by Dr. Cohn (10) it seems that while tuberin has some acid- and alkali-combining power over this range, its greatest reactivity is beyond these limits. Ingold (28) has concluded ''that tuberin, or at any rate that part coagulated by heat, has very little effect in buffering the sap.'' Therefore, a further statement by Dr. Cohn concerning this matter may be of interest. He

The significance of titration values in plant juice studies

In a non-acid juice the organic acids are largely in the form of salts which do not react with alkali. Figure 2 shows that there can be very little titratable acid at reactions above pH 5.5 in the case of oxalic and tartaric acids, above pH 6.0 in the case of malic, above pH 6.5 in succinic, and above pH 7.0 in citric acid. WILLAMAN (52) has stated that titration data on plant juices have little significance since "the acids occur as salts to a considerable degree and the titration gives no idea of the absolute quantity of acids present." Only when the initial reaction of a juice is well within the range of reactivity of the organic acids and their acid salts with alkali, as in the case of such plants as *Begonia* (40), the acid succulents (20), and many fruits, does the usual titration with alkali to pH 7.0 or 8.3 include any appreciable amount of the organic-acid compounds present. HEMPEL (20) and LEUTHARDT (32) have shown the quantitative relation between the initial reaction of a plant juice and the amount of free acid which is present, the percentage at any given reaction depending on the nature of the constituent acids. HAYNES and BROWN (19) apply this relation in estimating the salt content of apples from the titration and pH values of their juice.

Titration of a non-acid juice may often be an accurate measure of the phosphates, whose major zone of reactivity is between pH 5.5 and 8.0. MARTIN (33, 34) has shown a quantitative agreement between the concentration of phosphate in the tissues of the sunflower and the buffer values of the juice between pH 5.2 and 8.0. TEAKLE's data (48) also show a relation between the degree of buffering of wheat juice over the phosphate range and the actual phosphate content of the juice.

If pH 8.3 be taken as the end-point, titration includes also a trace of such compounds as asparagin. In order, therefore, to determine quantitatively the concentration of any constituent in the juice by titration, consideration must be given to the pH limits of its zone of reactivity and to those of other substances whose reaction ranges might overlap this zone. Otherwise, the so-called "total acidity" measurement becomes merely a measure of total buffer capacity between specified pH limits, as is generally

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says in a private communication: "There is, of course, combination of tuberin with acid and base over the physiological range, but in the case of this protein, as of most others, the largest number of reactive groups appear to have dissociation constants in the neighborhood of pH 3.3 and pH 9.7. . . . The titration curve suggests that tuberin is no exception to the general rule. Roughly, to pass from pH 4.5 to 8.5 required four parts of alkali, whereas to go from pH 8.5 to 10.5 required eight parts, and from pH 2.5 to 4.5 something over ten parts. In the case of the potato juice as a whole buffering is, of course, in part due to this protein, and in part to the phosphates and other weak acids that may be present."

recognized. It is not surprising that these determinations have been so frequently found to bear no consistent relation to the corresponding hydrogen-ion concentrations in plant juices (16, 26).

HEMPEL (20) has called attention to the fact that when aluminum salts of the organic acids are present, such as the aluminum malate in succulents, titration with alkali between pH 6.8 and 9.2 is complicated by the precipitation of the insoluble aluminum hydroxide. She thus explained the greater amount of alkali required to titrate the juice to pH 9.2 as compared to that required to bring it to pH 6.8. She has concluded that the presence of unknown substances "renders titration of plant juices to the phenolphthalein end-point uncertain or valueless."

Comparison of the points of inflection and characteristic buffer zones of the plotted titration curve of a plant juice with those of known buffer compounds may afford a means of identifying these constituents in the plant. The possibility of utilizing the characteristics of the curves of the common plant acids to identify them in mixtures is suggested by the work of AUERBACH and SMOLCZYK (4) and TÄUFEL and WAGNER (47). LEUTHARDT (32) has shown that the characteristics of the titration curves of apples, grapes, lemons, and tomatoes are in agreement with the nature of the constituent acids as determined by chemical analysis. He concludes, however, that when a buffer mixture contains more than one acid, it is generally impossible to identify the acids from the titration curve alone.

TAGUE (46) showed the value of the titration method in the identification and quantitative estimation of amino acids. Subsequently HARRIS (18), HIRSCH (21), and TÄUFEL and WAGNER (47) studied the titration curves of protein degradation products and pointed out their value in analyzing these compounds in mixtures. LEUTHARDT (32) attributed the point of inflection at pH 10.4 in the curve for *Mesembryanthemum* juice to the amide, glutamin, the presence of which he proved by chemical analysis. Similarly, asparagin has been identified in the juice of the etiolated wheat seedlings of the present investigation.

Several investigators have called attention to similarities between the form of some region of the titration curve of a plant juice and that of a known solution containing substances occurring in the juice. Thus HOAGLAND and DAVIS (22) pointed out the resemblance of the titration curve of *Nitella* sap between pH 5.2 and 8.0 to the corresponding region of a curve obtained with a mixture of the inorganic salts, including phosphates, that were found to occur in the sap. YOUDEN and DENNY (53) noted that the titration curve of apple juice was like that of a mixture containing its acid constituent, malic acid, and sodium malate. They also compared the curve for a mixture of organic acids with the curve for potato juice.

Titration values are now finding another use in quantitative estimations of buffer constituents of plant juices through comparisons of so-called buffer-index values. The slope of the tangent to the titration curve is a measure of the buffering power of the juice at any given point on the curve, and varies with the concentration of the substance being titrated. The buffer index constitutes a measure of the slope of the line connecting the two points on the titration curve which limit the region under consideration, or, the slope of the tangent at the intermediate point. The original unit, suggested by KOPPEL and SPIRO (29), is the amount of alkali or acid required to produce a unit change in the pH value of a solution, corrected for the amount required to produce the same change in the solvent alone. VAN SLYKE's unit (49) differs in not incorporating the correction for dilution, but, as this investigator points out, between pH 3 and 11 the correction is very small and the units are essentially the same. INGOLD (28) has shown the effectiveness of the VAN SLYKE buffer index as a means of determining the percentage of buffering in potato juice due to phosphates, citrates, and ether-soluble substances, respectively, over each unit pH range between pH 4.0 and 7.0.

For a further discussion of the significance of the titration curve and buffer-index concepts from the standpoint of quantitative analysis of plant juices, reference should be made to LEUTHARDT's paper (32).

Discussion

The question as to whether the principal buffers of the wheat plant have been identified and the buffer system approximately reproduced in proper concentration must remain problematical until complete analyses of the juice are available. However, it does not seem probable that the close agreement between the titration values of the buffer mixtures and those of the different juice samples is entirely fortuitous. It is of interest in this connection to compare the composition of these mixtures with what is known of the occurrence of their constituents in etiolated and in green wheat plants.

Asparagin.—Asparagin has frequently been reported to occur in wheat (9, 13, 14, 41a, 43, 44, 50). It is known to occur in greater amount in etiolated than in the green seedlings of many plants (37) including wheat (50) and other cereals (2, 8, 41, 45). So the fact that more asparagin was required to reproduce the curve for etiolated wheat seedlings than was used to reproduce that of green seedlings is consistent with the results of quantitative analyses. The curve for emergent seedlings, which, being partially etiolated, should contain an intermediate concentration of asparagin, was accurately reproduced on the basis of this assumption. The gradual decrease in the buffer capacity of the juice over much of the alkaline range

during the period of seedling development may therefore be assumed to reflect the utilization of asparagin in protein synthesis.

Organic acids.—The organic acids of the wheat plant have not been determined. On the basis of the form of the acid-titration curve of the juice, certain predictions may be made as to the organic-acid salts most likely to be present. Of the five commonest ones,—oxalates, succinates, tartrates, citrates, and malates,—the first two are at once eliminated by pronounced inflections below pH 4.0 (fig. 2). There is no trace of such an inflection in the curve for wheat juice. The titration curve of sodium malate is so similar to that of the juice over the acid range that it suggests that salts of this acid are the most abundant. However, fig. 2 shows that a similar curve is obtained with a combination of citrates and tartrates. It is apparent that a combination of malates, citrates, and tartrates would give practically the same curve. As LEUTHARDT (**32**, p. 30) has pointed out, it is probably impossible to identify with certainty the constituents in such a mixture by titration. However, it is obvious that salts of such acids as oxalic and succinic, having characteristically inflected curves, are not present in wheat in sufficient amounts to affect its titration values.

There is but little information available on the quantitative effects of etiolation on the acidity of any plant. Most investigators (**3, 17, 30, 38, 42**) who have worked on this problem have used titration methods which are uncertain for the present purpose, owing to the fact that phosphates and perhaps other buffers were included in the titrations. BASSALIK (**6**), however, has determined by quantitative analysis that etiolated plants of *Rumex, Oxalis* and *Begonia* contain less oxalic acid than do corresponding plants grown in the light. This finding is consistent with the fact that about three times as much sodium malate was required for the reproduction of the green-seedling curve of wheat as was used in duplicating the etiolated-seedling curve.

No data have been published, apparently, on changes in organic-acid content with seedling development. The progressive increase in the acid-combining power of the juice over the organic-acid buffer range during this period was reproduced by increasing the amount of sodium malate in the buffer mixture, suggesting the occurrence of a corresponding increase in such a buffer in the tissues as the young seedlings develop from the partially etiolated condition which characterizes them at emergence. Thus all the evidence of the titration curves is in agreement with BASSALIK's (**6**) report and with ASTRUC's (**3**) general conclusion that the formation of organic acids is greater in green tissues than in those without chlorophyll.

Phosphates.—ANDRÉ (**2**) reported that etiolation decreases the phosphate content of corn and lupin, but RISSMANN (**39**) found a somewhat higher concentration (as P_2O_5) in etiolated wheat seedlings than in the

corresponding tissues of the green plants. RISSMANN's results do not at first seem consistent with the fact that etiolated wheat seedlings have slightly less buffer capacity over the phosphate range than do green ones. However, etiolated plants have a higher water content than do plants grown in light (**15, 36,** etc.). The fact that RISSMANN's data' are based on dry weights of the tissues probably explains their failure to agree with the fact that the concentration of the phosphate in the juice of etiolated seedlings seems from the titration data to be a little lower than in the juice of green seedlings.[5]

Phosphates are evidently the most important buffers in wheat inasmuch as they regulate the hydrogen-ion concentration at the reaction of the juice. The pH value during the vegetative period of plants grown in soil under normal greenhouse conditions has been found to be near 6.0 (**23, 24, 27**). This same value is characteristic of etiolated seedlings also (**25**). The ordinary reaction limits for wheat have been found to be pH 5.5 and 6.3, although occasional measurements as low as 5.3 and as high as 6.4 have been obtained. The most acid juices (below 5.7) were obtained only in late maturation stages, or in vegetative stages when the plants were unhealthy. It is not improbable that the functioning of the phosphate equilibrium in the maintenance of the proper acidity is analogous to that of the carbonate equilibrium of the blood.

Summary

1. Both the alkali- and acid-titration curves of the juice of etiolated and of green wheat seedlings have been approximately reproduced with chemical mixtures containing asparagin, phosphates, sodium malate, glucose, and, in the case of green seedlings, leucin.

2. Asparagin appears to be the substance in the juice of the etiolated seedlings which is responsible for the characteristic point of inflection of the titration curve near pH 8.9.

3. Phosphates seem to be the principal buffers between pH 6.0 and 7.5. The equilibrium between the primary and secondary phosphates may be intimately associated with the maintenance of the normal reaction of the tissues.

4. Over the range of the acid-titration of the juice, *i.e.*, below pH 6.0, the buffering seems largely due to the presence of an organic-acid con-

[5] The average water content of the tissues and specific gravity measurements of the juice of week-old wheat seedlings grown under the conditions of the present investigation were as follows:

	H₂O (PER CENT.)	SP. GR.
Etiolated seedlings	93.7	1.014
Green seedlings	90.1	1.021

stituent. A solution of sodium malate alone, or a combination of tartrate and citrate, with or without malate, gives acid-titration values similar to those of the juice. The zone of decreasing buffer capacity below pH 3.5 and pH 4.0, respectively, in the oxalate and succinate curves, giving rise to characteristic inflections which do not occur in the titration curves of wheat juice, is believed to preclude the presence of these salts in wheat juice in sufficient amount to affect the titration values.

5. Glucose supplied the requisite buffer capacity of the etiolated-seedling juice above pH 9.5. In the case of green seedlings, both leucin and glucose were used in reproducing the titration curve over this region because the requisite amount of glucose alone was so much greater than that found in the juice. The glucose was considered to be representative of all the soluble carbohydrates of the juice which react with alkali above pH 9.5.

6. The most pronounced changes in the buffer system of the wheat plant during the period of seedling development seem, from the evidence presented in this paper, to be due to a reduction in asparagin content and an increase in an organic-acid constituent as assimilation processes become established in the young plant.

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LITERATURE CITED

1. ABBOTT, O. Chemical changes at beginning and ending of rest period in apple and peach. Bot. Gaz. **76**: 167–184. 1923.

2. ANDRÉ, G. Étude de quelques transformations qui se produisent chez les plantes étiolées à l'obscurité. Compt. Rend. Acad. Sci. Paris **130**: 1198–1201. 1900.

3. ASTRUC, A. Recherches sur l'acidité végétale. Ann. Sci. Nat. Bot. (Ser. 8) **17**: 1–108. 1903.

4. AUERBACH, FR., and SMOLCZYK, E. Zur Theorie und Praxis der electrometrischen Säuretitration. Zeitschr. physik. Chem. **110**: 65–141. 1924.

5. AUESBERGER, A. Pufferstudien II. Titerbestimmung von Pufferlösungen. Helv. Chim. Acta **9**: 823–826. 1926.

6. BASSALIK, C. Über die Rolle der Oxalsäure bei den grünen Pflanzen. I. Die Zersetzung der Oxalsäure bei *Rumex acetosa*. Bull. Intern. Acad. Sci. Cracovie **1916** (B): 203–240. 1917.

7. BJERRUM, N. Die Konstitution der Ampholyte, besonders der Aminosäuren, und ihre Dissoziationskonstanten. Zeitschr. physik. Chem. **104**: 147–173. 1923.

8. BOUSSINGAULT, J. B. De la végétation dans l'obscurité. Compt. Rend. Acad. Sci. Paris **58**: 917–922. 1864.

9. CHOATE, HELEN A.   Chemical changes in wheat during germination. Bot. Gaz. **71**: 409–425. 1921.

10. COHN, E. J.   The relation between the isoelectric point of a globulin and its solubility and acid-combining capacity in salt solution. Proc. Nat. Acad. Sci. **6**: 256–263. 1920.

11. ——————.   The activity coefficients of the ions in certain phosphate solutions.   A contribution to the theory of buffer action. Jour. Amer. Chem. Soc. **49**: 173–193. 1927.

12. ——————, GROSS, J., and JOHNSON, O. C.   The isoelectric points of the proteins in certain vegetable juices.   Jour. Gen. Physiol. **2**: 145–160. 1919.

13. ECKERSON, S. H.   Microchemical studies in the progressive development of the wheat plant.   Washington Agr. Exp. Sta. Bull. **139**. 1917.

14. FRANKFURT, S.   Zur Kenntnis der chemischen Zuzammensetzung des ruhenden Keims von *Triticum vulgare*.   Landw. Versuchs-Sta. **47**: 449–470. 1896.

15. GRANDSIRE, A.   Le chimisme des feuilles privées de chlorophylle.   Ann. Sci. Nat. (Ser. 10) **8**: 221–298. 1926.

16. GUSTAFSON, F. G.   Total acidity compared with actual acidity of plant juices.   Amer. Jour. Bot. **11**: 365–369. 1924.

17. HAAS, A. R. C.   Studies on the reaction of plant juices.   Soil. Sci. **9**: 341–368. 1920.

18. HARRIS, L. J.   The titration of amino- and carboxyl-groups in amino-acids, polypeptides, etc.   Pts. 1–3.   Investigations with aqueous solutions.   Proc. Roy. Soc. London (B) **95**: 440–484. 1923.

19. HAYNES, D., and BROWN, J. W.   A method for the estimation of the salt content from the pH value of apple juice, and some comparative analyses of the mineral content of the juice and whole apple. Biochem. Jour. **22**: 947–963. 1928.

20. HEMPEL, J.   Buffer processes in the metabolism of succulent plants. Compt. Rend. Trav. Lab. Carlsberg **13**: 1–129. 1916.

21. HIRSCH, P.   Neue Möglichkeiten der Acidimetrie, besonders zur Anwendung auf Eiweisskörper und deren Spaltungsprodukte.   Biochem. Zeitschr. **147**: 433–480. 1924.

22. HOAGLAND, D. R., and DAVIS, A. R.   The composition of the cell sap of the plant in relation to the absorption of ions.   Jour. Gen. Physiol. **5**: 629–646. 1923.

23. HURD, ANNIE M.   Hydrogen-ion concentration and varietal resistance of wheat to stem rust and other diseases.   Jour. Agr. Res. **23**: 373–386. 1923.

24. ———————. The course of acidity changes during the growth period of wheat with special reference to stem-rust resistance. Jour. Agr. Res. **27**: 725–735. 1924.

25. HURD-KARRER, ANNIE M. Acidity and varietal resistance of wheat to *Tilletia tritici*. Amer. Jour. Bot. **12**: 359–371. 1925.

26. ———————. Changes in the buffer system of the wheat plant during its development. Plant Physiol. **3**: 131–153. 1928.

27. ———————. Relation of leaf acidity to vigor in wheat grown at different temperatures. Jour. Agr. Res. **39**: 341–350. 1929.

28. INGOLD, C. TERENCE. The hydrion concentration of plant tissues X. Buffers of the potato tuber. Protoplasma **6**: 51–69. 1929.

29. KOPPEL, M., and SPIRO, K. Über die Wirkung von Moderatoren (Puffern) bei der Verschiebung des Säure-Basengleichgewichtes in biologischen Flüssigkeiten. Biochem. Zeitschr. **65**: 409–439. 1914.

30. KRAUS, G. Ueber die Wasservertheilung in der Pflanze. IV. Die Acidität des Zellsaftes. Abhandl. d. naturf. Ges. Halle **16**: 143–205. 1879.

31. LeCLERC, J. A., and BREAZEALE, J. F. Translocation of plant food and elaboration of plant material in wheat seedlings. U. S. Dept. Agr. Bur. Chem. Bull. **138**. 1911.

32. LEUTHARDT, F. Pufferkapazität und Pflanzensäfte. Kolloidchem. Beih. **25**: 1–68. 1927.

33. MARTIN, S. H. The hydrion concentration of plant tissues. IV. The buffer of sunflower hypocotyl. Protoplasma **1**: 522–536. 1927.

34. ———————. The hydrion concentration of plant tissues. VII. The buffers of sunflower stem and root. Protoplasma **3**: 273–281. 1928.

35. ———————. The hydrion concentration of plant tissues. VIII. The buffers of bean stem and root. Protoplasma **3**: 282–301. 1928.

36. PALLADIN, W. Transpiration als Ursache der Formänderung etiolierter Pflanzen. Ber. deut. bot. Ges. **8**: 364–371. 1890.

37. PALLADIN, V. I. Plant Physiology. [Eng. Ed. Trans. by Livingston.]

38. PURJEWICZ, K. Die Bildung und Zersetzung der organischen Säuren bei den höheren Pflanzen. Kiew, 1893 [Russian] Rev. in Bot. Centralb. **58**: 368–374. 1894.

39. RISSMANN, R. Der Mineralstoffwechsel grüner und etiolierter Pflanzen unter besonderer Berücksichtigung des Magnesiums und der Chlorophyllbildung. Zeitschr. wiss. Biol. Abt. E., Planta **9**: 195–245. 1929.

40. Rose, Dean H., and Hurd-Karrer, Annie M. Differential staining of specialized cells in *Begonia* with indicators. Plant Physiol. **2**: 441–453. 1927.

41. Schulze, E., and Kisser, E. Über Zersetzung von Proteinstoffen in verdunkelten grünen Pflanzen. Landw. Versuchs-Sta. **36**: 1–8. 1889.

41a. Schulze, E. Über die chemische Zusammensetzung der Samen unserer Kulturpflanzen. Landw. Versuchs-Sta. **73**: 35–170. 1910.

42. Steinmann, A. B. Studien über die Azidität des Zellsaftes beim Rhabarber. Zeitschr. Bot. **9**: 1–59. 1917.

43. Stieger, A. Untersuchungen über die Verbreitung des Asparagins, des Glutamins, des Arginins und des Allantoins in den Pflanzen. Hoppe-Seyler's Zeitschr. physiol. Chem. **86**: 245–269. 1913.

44. Suzuki, U. On the formation of asparagin in plants under different conditions. Bull. Coll. Agr. Tokyo **2**: 409–457. 1896.

45. ——————. On the formation of asparagin in the metabolism of shoots. Bull. Coll. Agr. Tokyo. **4**: 351–356. 1902.

46. Tague, E. L. A study of the determination of amino acids by means of the hydrogen electrode. Jour. Amer. Chem. Soc. **42**: 173–184. 1920.

47. Täufel, K., and Wagner, C. Über die Bedeutung und die Ermittlung der potentiellen Acidität. Zeitschr. angew. Chem. **40**: 133–140. 1927.

48. Teakle, L. J. H. The absorption of phosphate from soil and solution cultures. Plant Physiol. **4**: 213–232. 1929.

49. Van Slyke, D. D. On the measurement of buffer values and on the relationship of buffer value to the dissociation constant of the buffer and the concentration and reaction of the buffer solution. Jour. Biol. Chem. **52**: 525–570. 1922.

50. Waśniewski, S. Der Einfluss der Temperatur, des Lichtes und der Ernährung mit Stickstoff und Mineralstoffen auf den Stoffwechsel in den Keimpflanzen des Weizens. Bull. Intern. Acad. Sci. Cracovie. (B) **1914**: 615–686. 1915.

51. Wehmer, C. Die Pflanzenstoffe. Jena. Gustav Fisher. 1911.

52. Willaman, J. J., West, R. M., Spriesterbach, D. O., and Holm, G. E. Notes on the composition of the sorghum plant. Jour. Agr. Res. **18**: 1–31. 1919.

53. Youden, W. J., and Denny, F. E. Factors influencing the pH equilibrium known as the isoelectric point of plant tissue. Amer. Jour. Bot. **13**: 743–753. 1926.

THE PHYSIOLOGY OF *CONVOLVULUS ARVENSIS* (MORNING-GLORY OR BINDWEED) IN RELATION TO ITS CONTROL BY CHEMICAL SPRAYS

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(WITH FIVE FIGURES)

I. Introduction

(a). The reaction of plants to chemical sprays, and the effectiveness of these sprays, depend upon penetration and subsequent distribution of the chemical throughout the living tissues. This paper reports some experiments relative to the factors which control these processes in the wild morning-glory (*Convolvulus arvensis*).

Early writers (3, 5) on morning-glory control prescribe clean cultivation, pasturing, and the use of smother crops. That these methods were unsuccessful in the hands of practical farmers was recognized in California in 1915; and in the fall of that year GEORGE P. GRAY (8) started a series of experiments on the use of chemical sprays. A number of plant poisons were used but the application of sodium arsenite solution to the leaves of mature plants was the only treatment which he found successful. Recommendations were made (7) for the use of this spray in the coastal fog belt of California, but it was not extensively used by farmers. The materials were dangerous to handle and the treatment allowed the maturing of a crop of seed before the application of the spray, making eradication practically impossible.

GRAY proved that the perennial roots of the morning-glory could be killed by the application of arsenic to the leaves. He realized the importance of this fact and emphasized the need for further study of the problem as the mechanism responsible for the distribution of the arsenic within the plant was not understood at that time.

Few of the workers after GRAY have followed his suggestion. Publications from the United States Department of Agriculture (17), California (2), Utah (16), and Colorado (14), adhere to the older recommendations. The Colorado workers (14) found no evidence for translocation of arsenic in their experiments with K.M.G.[1] Better results were obtained with this product in Washington (15) but it was not completely satisfactory.

The present study was started in 1925 for the purpose of determining the mechanism responsible for the translocation of arsenic within the morning-glory plant. Continued attempts to control this weed by cultiva-

[1] K.M.G. is a solution of AsCl₃ having an acid reaction and is recommended for the control of morning-glory by the Weed Control Company of California, Berkeley.

tion methods had failed and chemicals seemed to offer the most promise. The senior author took up this work in 1926 under the late Dr. P. B. KENNEDY and published with him in 1927 (12) a short paper, suggesting a possible mechanism and enumerating the principal factors which tend to limit its action. JOHNSON has since suggested a somewhat different mechanism based upon an idea of GRAY's (8, p. 95). The evidence presented in the present paper should aid in clarifying the problem with respect to the translocation of arsenic when it is applied to the leaves of mature plants.

(b). The killing of plants by toxic sprays depends upon two factors, namely, penetration and distribution. Movement of the spray solution from the leaf surface into the vascular strands is hindered by the cuticle and by the living cells of the mesophyll. This hindrance is most effectively overcome by the incorporation in the spray solution of acids or bases which hasten diffusion through the cuticle, and rapidly kill the underlying cells. Insect injuries often aid materially in allowing entry through the cuticle. Distribution, on the other hand, is effected through the natural conducting systems of the plant and is dependent upon the internal conditions at the time of spraying. The experimental work on this problem has led to the conclusion that water movement in the morning-glory takes place in accordance with the scheme proposed by DIXON (6). When the plant is actively transpiring, pressure within the xylem is reduced; the vessels, being elastic, are partially compressed, resulting in a decreased capacity, and a water deficit is set up in all of the living cells of the plant. If a solution under atmospheric pressure is released into the xylem in this condition, the sudden expansion of the vessels will cause a very rapid uptake until the vessels have attained their normal capacity. Following this a slower osmotic absorption by the living cells will continue until all deficit is satisfied. Therefore, when an extremely toxic spray is applied to the leaves and stems of a morning-glory plant and the cortical tissues rapidly killed, the sap which they contain, mixed with more or less of the toxic spray solution, will pass into the xylem vessels and be forced down toward the roots. The extent of movement will depend upon how completely the original xylem sap is removed and replaced by the descending current of solution. Instances have been observed where eosin was carried to within 20 centimeters of the growing points of roots during an exposure of one hour. In such extreme cases as these there may even have been some loss of water from the roots into the surrounding soil.

It is difficult to picture the downward movement of arsenic through the phloem, with the organic nutrients from the leaves, as suggested by GRAY (8) and JOHNSON (11), since this tissue is composed wholly of living cells in an active state. Such an active poison would rapidly kill these cells and

render the system functionless for conduction. For the same reason the latex system which may be auxiliary to the phloem in the conduction of organic nutrients (10) cannot be considered as effective in the transfer of arsenic.

The persistent vegetative activity of the morning-glory plant is made possible by the storage of relatively large quantities of starch in the root system. The rapidity with which these reserves may be depleted and replenished indicates the presence of a very efficient conducting mechanism. The structure of the latex vessels (13) indicates that at least in the storage process they may aid materially in conduction. The vessels extend from the leaves into the primary root tips and latex will exude from them wherever they are punctured. It may be forced from the vessels in short lengths of stem by means of air pressure and flows from the severed branches into water in relatively large quantities, especially if they are well illuminated. Pressure in the latex vessels seems lowest in dormant roots and highest in young actively growing tissues.

Any treatment which reduces the photosynthetic activities of the plant tends also to reduce starch reserves, but in order to be effective in control, the depletion must be complete. Elimination of top growth for one season has not always been successful, and similar treatment for three years did not eradicate an old stand in California.

Two kinds of chemical sprays have proved effective in morning-glory control, namely, arsenicals and the alkali chlorates. Only the former will be considered in this paper. The results obtained with chlorates will be left for consideration in a later publication.

II. The toxic action of arsenical sprays

A. Penetration

1. *The reaction of the spray solution.*—In order that the above pictured mechanism may function effectively in the distribution of arsenic throughout the plant, the spray solution must perform two functions. First, it must penetrate the cuticle and rapidly kill the cortical tissues, allowing the arsenic to enter the vascular strands; and second, it must supply arsenic in such a concentration that, even after considerable dilution, a lethal dose is delivered to all living tissues of the root. Acid (1, 4) and alkali are among the cheapest and most effective materials for killing plant tissues. The results of a comparative test of several common agents are given in figures 1, 2, 3, and 4. In figs. 1 and 2 injury is plotted against exposure time, in figs 3 and 4 injury is plotted against time, the exposure time being noted on the curves. These records are of visual injury occurring to young morning-glory shoots allowed to hang in the solutions for the given "ex-
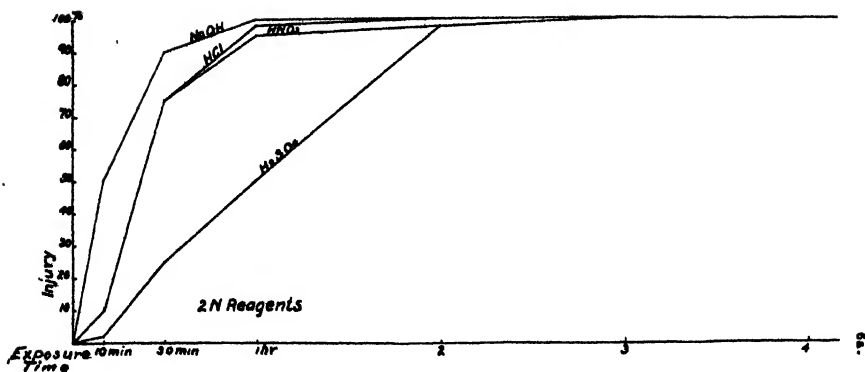
FIG. 1. Injury by reagents, 2N strength, with exposure time.
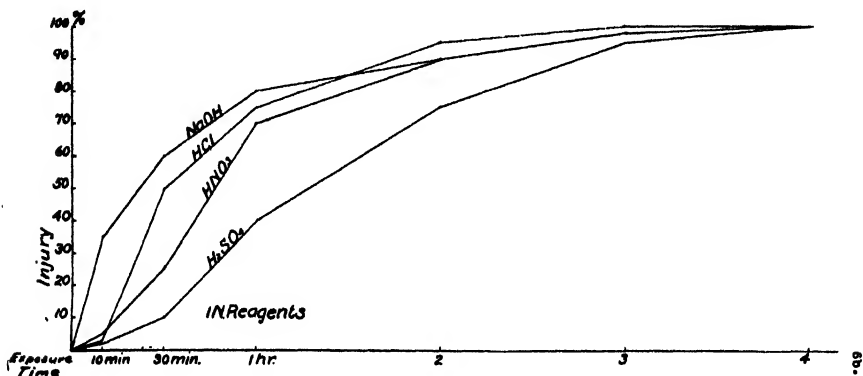
FIG. 2. Injury by reagents, 1N strength, with exposure time.
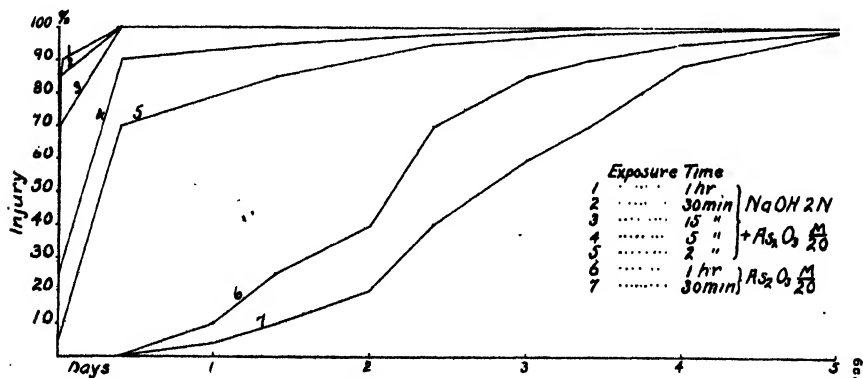
FIG. 3. Injury with alkali and arsenious oxide, with time.
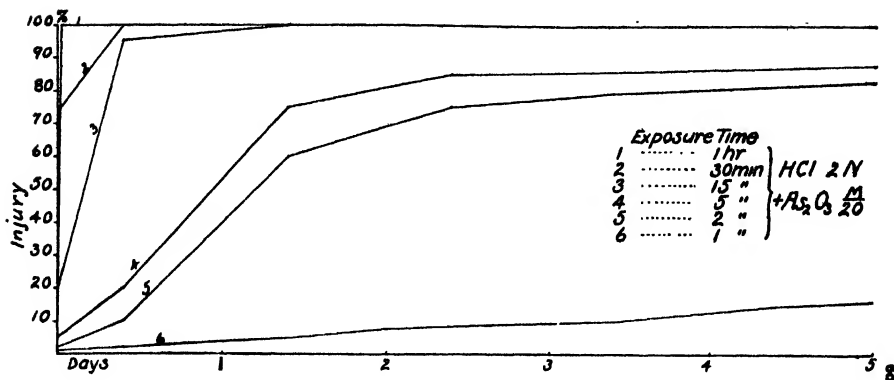
FIG. 4. Injury with acid and arsenious oxide, with time.

posure time'' and then removed, washed in tap water and set up in flasks of tap water to allow the injury to develop. The cut ends were protected from the solutions by paraffin coatings and kept above them during the exposure. Under these conditions NaOH proved the most toxic, HCl and HNO$_3$ were about the same, and H$_2$SO$_4$ was less toxic. The addition of $\dfrac{M}{20}$ As$_2$O$_3$ did not affect the initial toxicity of these agents and in neutral solution it acted very slowly upon the tissues. The acid solutions caused a light brown discoloration and the basic solutions a darkening of the green color.

To determine the pH range in which acids and bases are effective, morning-glory shoots were set up in flasks and allowed to absorb through their cut ends arsenic solutions in concentrations of $\dfrac{M}{20}$, $\dfrac{M}{80}$, $\dfrac{M}{320}$, $\dfrac{M}{1280}$, and $\dfrac{M}{5120}$, each concentration including solutions having pH values of 1, 3, 5, 7, 9, and 11. The solutions were buffered with phosphate and the pH values adjusted daily. The results indicated that solutions lying between pH 3 and pH 9 do not cause immediate injury due to their pH value. Injury due to arsenic was evident after 20 hours and in the three higher concentrations it was complete within 48 hours. It was necessary for the shoots in the two weaker arsenic concentrations to accumulate arsenic in the leaves before injury became apparent. A similar test using K.M.G., AsCl$_3$, Na$_2$HAsO$_3$, and As$_2$O$_3$ in comparable concentrations showed that the same conclusions applied to these chemicals. The progress of acid injury in the stems standing in the pH 1 solutions was slow compared with the rise of arsenic or eosin solutions. This indicates that the plant buffers are able to neutralize hydrogen-ion while the arsenic continues to rise in the stems.

Plants growing in the greenhouse in soil tubes were sprayed with solutions of 1N HCl, 1N NaOH, $\frac{M}{20}$ As$_2$O$_3$ in neutral solution and with 1N HCl and NaOH solutions containing $\frac{M}{20}$ As$_2$O$_3$. The acid and alkaline solutions rapidly killed those tissues with which they came in contact, the neutral solution of As$_2$O$_3$ slowly killed the tissues to which it was applied, but the solutions containing acid or alkali plus arsenic killed the root tissues to some depth below the place of application. As$_2$O$_3$ has a maximum concentration of about $\frac{M}{20}$ in neutral saturated solution. The fact that this is approximately the optimum concentration for arsenic in a spray solution indicates that more arsenic would be of no avail after the plant buffers had neutralized the acid or alkali of the spray.

Comparison of NaOH, HCl, and H$_2$SO$_4$ in arsenic solutions applied in field trials at Davis has shown that the acid solutions are the most effective. When the concentration of alkali becomes great enough to cause rapid killing, its softening action upon the plant tissues causes them to collapse and the absorption of the arsenic is hindered.

B. DISTRIBUTION

1. *Water relations of the plant as affecting the movement of arsenic.*—In studying the movement of arsenic through xylem tissues it was necessary to ascertain whether or not adsorption phenomena or other chemical reactions with the surrounding walls or cells would interfere with its passage through the narrow channels. Many basic dyes are adsorbed to lignified walls, certain inorganic acids react with buffers in the tissues, and oxidizing agents may be reduced in their passage through xylem vessels. A set of preliminary tests using acid, basic and neutral solutions of arsenic in conjunction with eosin and other dyes showed that arsenic either in an anion or as a cation moved readily through morning-glory stems and roots without being rapidly withdrawn from the solution. In table I the time required for arsenic injury to become visible after reaching the tissues is shown. In this experiment morning-glory shoots were set up in arsenic solutions of the concentration given in the first column of table I. Comparable sets in eosin solution were used to indicate the rate of ascent of the transpiration current. The chief interest in this data lies in the close correlation between transpiration and the occurrence of arsenic injury. Since it takes about twenty hours for enough cells to die and become sufficiently discolored to produce visible signs of injury under these conditions, the increase in transpiration due to the breeze from an electric fan had no

appreciable effect on the shoots in the higher concentrations; but on those in the lower ones which required accumulation in the leaves to reach a lethal concentration, a marked effect may be noted.

TABLE I

EFFECT OF TRANSPIRATION UPON THE MOVEMENT OF ARSENIC INTO MORNING-GLORY SHOOTS

As_2O_3 CONC.	LIGHT BREEZE		OPEN ROOM		MOIST CHAMBER	
	EOSIN[1]	ARSENIC[2]	EOSIN[1]	ARSENIC[2]	EOSIN[1]	ARSENIC[2]
$\frac{M}{20}$	$\frac{1}{2}$ hr.	24 hrs.	1 hr.	24 hrs.	24 hrs.	48 hrs.
$\frac{M}{80}$	$\frac{1}{2}$ hr.	24 hrs.	1 hr.	24 hrs.	24 hrs.	72 hrs.
$\frac{M}{320}$	$\frac{1}{2}$ hr.	34 hrs.*	1 hr.	34 hrs.	24 hrs.	72 hrs.*
$\frac{M}{1280}$	$\frac{1}{2}$ hr.	48 hrs.	1 hr.	72 hrs.	24 hrs.	108 hrs.
$\frac{M}{5120}$	$\frac{1}{2}$ hr.	72 hrs.	1 hr.	168 hrs.	24 hrs.	No injury

[1] Time for complete filling of the vascular system.

[2] Time for appearance of arsenic injury.

* Only 4 shoots were subjected to each treatment and the large experimental error renders these data only roughly quantitative.

The period of twenty to twenty-four hours required for arsenic injury to become visible may have been required for accumulation to a lethal concentration, or the injury may simply have been due to a slow reaction. Eosin will kill the leaves in about four hours so this time is not all required for diffusion. Table II gives data on an experiment designed to clarify this point. Shoots were gathered, allowed to stand in tap water in the laboratory for two hours, and the cut ends placed in a $\frac{M}{20}$ As_2O_3 solution. At definite intervals sets of four shoots were removed, washed under the tap, and set up in flasks containing water. The first row of table II gives the time intervals during which the sets were allowed to absorb arsenic solution. The percentage of injury was estimated visually. Sufficient arsenic to completely kill the tissue was being absorbed within one hour, but injury was apparent only after about twenty hours. The injury to the shoots which had only a short time to absorb was different from the usual type, appearing first in the young tender stems just below the tips, spreading down the stem and out the petioles and mid-ribs, and affecting the mesophyll to the least extent. This is typical of injury by very dilute solutions and indicates that the vascular tissues are more susceptible than mesophyll.

This agrees with the fact that the lethal concentration in leaves is higher than in roots where the parenchyma of the vascular system is the first tissue to show injury.

TABLE II

ARSENIC INJURY TO MORNING-GLORY SHOOTS WHICH WERE ALLOWED TO ABSORB $\dfrac{M}{20}$ As$_2$O$_3$

SOLUTION FOR VARYING PERIODS OF TIME

ABSORPTION TIME	ARSENIC INJURY			TISSUES CONTAINING EOSIN*
	AFTER 24 HOURS	AFTER 29 HOURS	AFTER 42 HOURS	
hours	per cent.	per cent.	per cent.	per cent.
0.25	0	0	25	25
0.50	0	25	100	50
0.75	50	50	100	75
1.0	75	100	100	100
1.5	75	100	100	100
2.0	100	100	100	100
2.5	75	75	100	100
3.0	75	100	100	100
4.0	75	100	100	100
5.0	100	100	100	100
6.0	100	100	100	100
8.0	100	100	100	100
17.0	100	100	100	100
24.0	100	100	100	100

* Estimated visually: after two hours there was a visible accumulation of eosin in the tissues; after four hours the tissues were injured; and after five hours death occurred.

Since eosin and arsenic solutions move unhindered through xylem tissues, if stems are cut under these solutions, the rate of uptake should be an index of the amount of water deficit existing in the plants at the time the stems are cut. In table III rates of eosin uptake by stems are recorded for plants which were growing under different conditions of soil moisture, humidity, temperature, and illumination. The extent of movement was determined by removing the cortical tissues and observing the stained xylem.

Rates measured in roots proved even greater, the xylem vessels being larger. The effect of air in the vessels was shown by a series of tests made by cutting the stems in air and immediately immersing them in the eosin solution. The stems were on the same plants as those which gave the rate of 2.46 inches per second when cut under the solution and twenty-six of them gave an average rate of 0.9 inches per second. The larger vessels

TABLE III

RATES OF FLOW OF EOSIN SOLUTIONS INTO MORNING-GLORY STEMS CUT UNDER SOLUTION. (MOVEMENT RECORDED FROM CUT END TOWARD CROWN OF PLANT)

PLACE	PART OF PLANT AND GROWING CONDITIONS	TIME	TEMPERA-TURE	HUMIDITY	EXPOSURE	NUMBER OF TESTS	RATE, INCHES PER SECOND
			° F.	per cent.	seconds		
At Berkeley:							
Laboratory	Potted plant from greenhouse		70		30	3	0.18
Laboratory	Plant in water culture solution		70		300	5	0.00
Greenhouse	Potted plant in greenhouse; soil dry		90		10	4	0.82
Greenhouse	Potted plant in greenhouse; soil dry		90		10	9	0.75
Greenhouse	Plant in water culture		90		10	4	0.70
Greenhouse	Plant in water culture; petiole cut and exposed		90		30	1	0.11
Field	Plant in moist soil; petiole cut and exposed		70		30	1	0.40
At Davis:							
Field	Mature plants in dry soil	2:30–3:00 P. M.	83	26	5	30	2.42
Field	Mature plants in dry soil	5:15–5:30 P. M.	80	40	5	20	2.46
Field	Mature plants in dry soil	7:30–8:00 P. M.	69	56	5	20	1.86
Field	Mature plants in dry soil	5:30–6:00 A. M.	48	70	5	20	1.26
Field	Plants in blossom, in dry soil	4:15–4:30 P. M.	81	38	5	16	1.53
Field*	Mature plants in wet soil	4:45–5:00 P. M.	80	40	5	10	1.64
Field*	Mature plants in wet soil	7:00–7:30 P. M.	71	55	5	25	0.98
Field*	Mature plants in wet soil	6:00–6:15 A. M.	76	53	5	20	0.45

* These plants were growing in a plot which had been irrigated the day before these tests were made.

were filled with air and this movement was taking place through the smaller ones which, due to the fact that their menisci were able to withstand the pressure difference which existed at the time the cut was made, had not lost their liquid contents.

This rapid uptake of a solution under the conditions of the experiment shows that a sub-atmospheric pressure exists in the xylem of morning-glory plants which are transpiring. That the water deficit thus developed is capable of causing a thorough distribution of a solution through the roots is indicated by the fact that when the tops of plants were cut off at the ground level under eosin the roots were filled to a depth of seven feet within one hour; and if arsenic solutions were used the roots were killed to a depth of seven feet or more (12).

The effect of soil moisture upon the depth of penetration of arsenic was shown by an experiment using plants growing in soil tubes in the greenhouse. Three series were used, the first having been watered daily, the second having been left for five days without being watered, and the third having the tubes containing the roots and surrounding soil submerged in water for one hour before the spray was applied. Four solutions were used as shown in table IV and the extent of injury is given, the roots having been removed three weeks after the spray was applied.

TABLE IV

DEPTH OF INJURY TO SPRAYED PLANTS

SOLUTION	SOIL NORMAL	SOIL DRY	SOIL SATURATED
HCl 1.6 N	Stems killed to crown	Stem killed to crown	Stems killed to crown
$As_2O_3 \frac{M}{20}$ in HCl 1.6 N	Stem killed. Root killed 4″ below crown	Stems killed. Roots killed 12″ below crown	Stems killed to crown
$As_2O_3 \frac{M}{20}$ in NaOH 0.5 N	Stems killed. Roots killed 2″ below crown	Stems killed. Roots killed 6″ below crown	Stems killed to crown
$As_2O_3 \frac{M}{20}$ pH 7.	Stems killed to crown	Stems killed to crown	Stems killed to crown

Interpretation of a large amount of field data on spray tests leads to the same conclusion as this experiment, namely, that the dryer the soil, other conditions being equal, the deeper will the arsenic penetrate.

2. *The effect of exposure time upon the extent of injury.*—If the spray solution does not have time enough to penetrate to the vascular tissues before it becomes dried on the leaves it cannot be carried to the remote parts

of the plant.   GRAY experienced this difficulty and could only obtain results (17) in the fog belt. Spraying in the afternoon when transpiration is at its height should favor deep penetration; and repeated spraying with water, following the initial application of arsenic, should avoid the drying difficulty.   The two plants shown in figure 5 were sprayed with an acid solution of arsenic, plant A being allowed to dry and plant B being kept wet for two hours following the initial application. These plants had young foliage and were growing in a wet field so that distribution of the arsenic was not thorough in either of them, but the effect of a long exposure time is apparent in plant B despite the lower top: root ratio in this plant.   The manufacturers of K.M.G. recommended that applications be made after sundown and during the night. Two plots of heavily infested land were used to test the effects of evaporation.   One was sprayed in the afternoon, and followed by applications of water which kept the plants wet for one hour after the initial treatment; the other plot was sprayed after dark. Examination of the plants two weeks later showed no apparent difference in the effectiveness of the treatments.   Evidently the night spraying did nothing except extend the exposure time. The advantage gained by the use of sulphuric acid in arsenic sprays, while partially due to the rapid killing and hardening of the plant tissue may also depend upon its hygroscopic nature.

C. ARSENIC CONTENT OF SPRAYED PLANTS

Quantitative analyses were carried out on twenty-seven different morning-glory plants which had been sprayed with arsenic solutions. The plants were dug about ten days after spraying, the roots washed under the tap, and, after evaporation of surplus water, samples were taken and dried at 80°.   These plants were growing in Berkeley and their roots branched at a depth of two feet or less and did not penetrate deeper than four feet. Table V shows the distribution of arsenic in the roots, being expressed as percentage As_2O_3 in the dry weight of a sample.

Twenty samples of stems and leaves gave an average percentage composition of 0.2011 per cent. As_2O_3. The concentration below which no injury had been noted in the fresh sample was 0.0003 per cent. in the roots and 0.02 per cent. As_2O_3 in the tops. This latter concentration was not determined on sprayed plants but on tops which had absorbed arsenic through branches cut under arsenic solution.

III. Discussion

The difficulties which caused GRAY (7) to limit the use of his sprays to the fog belt in his recommendations can be understood in the light of the
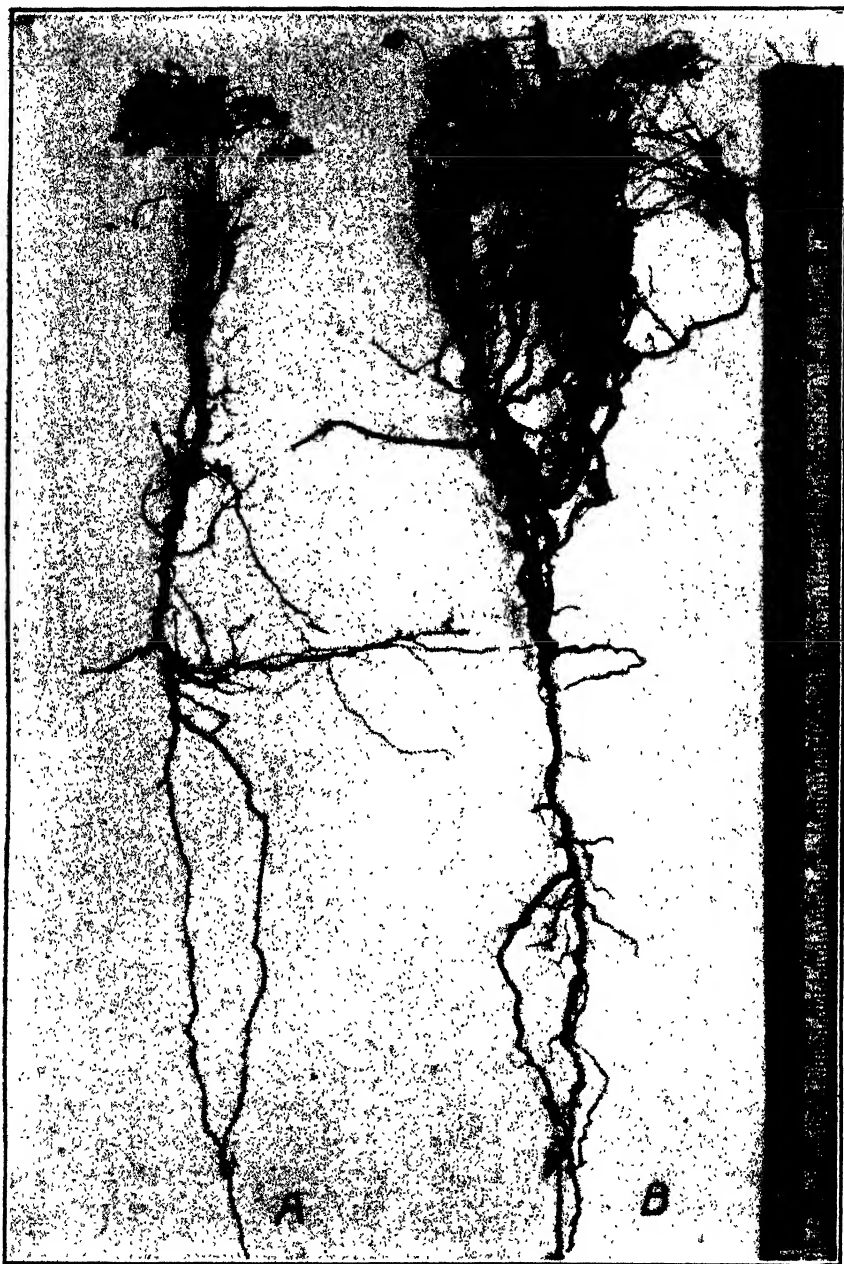
FIG. 5.   Two plants sprayed with a solution having 16 parts of water to one of K.M.G.
A. Plant allowed to dry after spraying.   As₂O₃ content 0.041 mg.   B. Plant
kept wet for two hours by repeated spraying with water.   As₂O₃ con-
tent 0.705 mg.   Injury present to a depth of 12 inches.

TABLE V
ARSENIC DISTRIBUTION IN MORNING-GLORY ROOTS

DEPTH IN GROUND	As$_2$O$_3$ CONTENT, DRY WEIGHT BASIS
inches	*per cent.*
2	0.00508
2 to 4	0.00448
4 to 6	0.00422
6 to 8	0.00302
8 to 10	0.00262
10 to 12	0.00231
12 to 15	0.00112
15 to 18	0.00044
18 to 24	0.00030
24 to 30	0.00001

foregoing experiments. Using an alkaline solution, if he increased the alkalinity to get more rapid penetration, the gelatinizing action of the free base caused clogging of the conducting channels. If he increased the arsenic he was soon limited by its solubility in the neighborhood of neutrality. The advantage of maturity of the plants was probably more closely related to water deficit than to downward transport of organic nutrients.

The benefits of an acid solution have been pointed out. The most effective arsenicals thus far tried have had an acid reaction. The use of arsenic as a toxic agent, however, is attended by certain inherent difficulties. Besides its low solubility at the pH of plant sap it requires the use of a killing agent to effect penetration of the cortical tissues. This agent rapidly destroys the mechanism responsible for the water deficit necessary for the transport of the arsenic. Once the spray is applied and the reaction of the plant has taken place, if the treatment has not been successful nothing more can be done during that season. Plants injured to a depth of a foot or more but not completely killed seem more resistant to further treatments the following season than do untreated plants.

The poisonous nature of arsenic necessitates its transport through non-living channels in lethal concentrations. This practically limits its movement within the plant to the xylem and prescribes a high water deficit and particularly favorable conditions for application of the spray. These conditions are seldom met in actual practice.

The latest developments in spraying technique take advantage of the water naturally occurring in the plant. Application of spray to the crown and stems allows early penetration in these regions. Subsequent spraying of the leaves releases a relatively large volume of sap into the xylem vessels resulting in a deep injection of the solution which has already

entered through the stems and which is consequently at the forward rather than at the rear end of the moving water columns. A thorough wetting of the plants is important also, for as soon as the available liquid has moved down into the roots all action ceases and the effectiveness of the treatment is fixed.

From a practical standpoint the use of arsenic as a herbicide has several advantages. It is cheap and easily obtained, it may be readily dissolved by strong reagents and later diluted for field use, and it has proved successful at least in some instances. The fact that its transport is limited to the xylem, however, almost precludes the possibility that its use can ever result in complete eradication of morning glory, for the conditions required for its thorough distribution do not occur until after the maturity of the first seeds. Possibly it can be used in some stages of a control program.

IV. Summary

1. The persistent vegetative activity of the morning-glory is related to the large storage of starch in its roots. Early control methods, based on an attempt to deplete this starch reserve, have not proven practical.

2. Killing of plants by arsenicals depends upon two factors: (a) penetration, and (b) distribution.

3. Acid solutions are more effective than basic ones in penetrating cortical tissues in the morning-glory.

4. Solutions lying between pH 3 and pH 9 do not cause rapid injury to tissues, the direct effect of their pH value being negligible.

5. Plant buffers are active in neutralizing acid and alkaline sprays.

6. Arsenic trioxide is soluble to a concentration of about one-twentieth molal at the pH of plant sap.

7. Arsenic moves freely through the xylem of morning-glory plants. Cut shoots may take up enough $\frac{M}{20}$ As$_2$O$_3$ solution in one hour to be completely killed. Injury appears only after a lapse of about 20 hours. Eosin may enter cut stems and move toward the roots at a rate as high as 2.46 inches per second for five seconds. The rate decreases as water deficit becomes satisfied.

8. Water deficit, as indicated by the uptake of eosin solution, is correlated with soil moisture and transpiration.

9. Prolonged exposure to the spray solution as by the condensation of dew or addition of water to the leaves greatly increases the depth of penetration of the arsenic.

10. The hygroscopic properties of sulphuric acid and its hardening effect upon tissues make it an effective ingredient of the spray solution.

11. Maturity is an index of the proper time for spraying only as it is accompanied by water deficit. An examination of the pressure conditions in the xylem is a much more accurate test.

12. The lethal concentrations of arsenic were 0.02 per cent. of the dry weight in tops and 0.0003 per cent. in roots.

13. Arsenic has been used with some success in the control of morning-glory but complete eradication is seldom accomplished by its use.

Acknowledgments

Most of the experimental work described in this paper was done by the senior author under the supervision of the late Dr. P. B. KENNEDY, and the original manuscript was prepared in conjunction with him. The present paper, however, has been entirely revised and rewritten since his death and so, while acknowledging his valuable guidance and aid throughout the course of the work, I assume personal responsibility for all of the material presented. I wish to express my gratitude to Dr. J. P. BENNETT for constant assistance in an advisory way as well as in the preparation of the manuscript, and to Professors B. A. MADSON and W. W. ROBBINS who have helped on several occasions.—A. S. C.

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LITERATURE CITED

1. ÅSLANDER, ALFRED. Sulphuric acid as a weed spray. Jour. Agr. Res. 34: 1065–1091. 1927.

2. BARNUM, C. C. The control of wild morning-glory. California Agr. Exp. Sta. Cir. 256. 1923.

3. BIOLETTI, F. T. The extermination of wild morning-glory. California Agr. Exp. Sta. Cir. 69. 1911.

4. BROWN, J. G., and STREETS, R. B. Sulphuric acid spray: A practical means for the control of weeds. Arizona Agr. Exp. Sta. Bull. 128. 1928.

5. COX, H. R. The eradication of bindweed or wild morning-glory. U. S. Dept. Agr. Farmers' Bull. 368. 1909.

6. DIXON, H. H. Transpiration and the ascent of sap in plants. Macmillan Co., Ltd. London. 1914.

7. GRAY, GEORGE P. Spraying for the control of wild morning-glory within the fog belt. California Agr. Exp. Sta. Cir. 168. 1917.

8. ——————. Tests of chemical means for the control of weeds. Univ. California Pub. in Agr. Sci. 4: 67–97.

9. ——————. Herbicides. California State Dept. of Agr. Monthly Bull. 11: 263–269. 1922.

10. HABERLANDT, G. Physiological plant anatomy. Translation from the fourth German edition by M. DRUMMOND. Pp. 336–345. Macmillan Co., Ltd. London. 1914.

11. JOHNSON, ETHELBERT. Recent developments in the use of herbicides in California. California State Dept. of Agr. Monthly Bull. **17**: 7–16. 1928.

12. KENNEDY, P. B. and CRAFTS, A. S. The application of physiological methods to weed control. Plant Physiol. **2**: 503–506. 1927.

13. ——————. The anatomy of morning-glory (or bindweed) (*Convolvulus arvensis*) in relation to its control by chemical sprays. Unpublished.

14. ROGERS, CHARLES F., DURRELL, L. W., and DANIELS, LESLIE B. Three important perennial weeds of Colorado. Colorado Agr. Exp. Sta. Bull. 313. 1926.

15. SCHAFER, E. G. The bindweed. Washington Agr. Exp. Sta. Popular Bull. 137. 1927.

16. STEWART, GEORGE, and PITTMAN, D. W. Ridding the land of wild morning-glory. Utah Agr. Exp. Sta. Bull. 189. 1924.

17. U. S. Dept. Agr. Wild morning-glory, or bindweed. U. S. Dept. Agr. Weed Investigations. April 1, 1921. (Information Circular 128, Division of Agronomy, University of California, Berkeley.)

DETERMINATION OF THE PERCENTAGE OF SUCROSE IN SUGAR BEETS FOR RESEARCH PURPOSES

DEAN A. PACK[1]

(WITH TWO FIGURES)

Introduction

Recently an article was published by REED (9), the purpose of which was: "To point out a source of error in the methods now used in sugar-beet testing and to present a method in which this particular error will not be possible."

The cold water digestion method is the one with which this author finds fault. He reports evidence of its inaccuracy and explains that, since all the cells containing sucrose are not broken when pulp samples are taken, complete extraction cannot be expected with the use of cold water. He proposes a method in which the principal innovation is that the sample, either as pulp or as chunks taken with a knife, with the necessary lead acetate added, is steamed at 15 pounds pressure for 15 minutes. The purpose of the autoclaving is to render all of the cells in the sample permeable to sucrose. Working with this autoclave method he reports that all the samples taken from an individual beet gave the same reading.

The cold water process is of such general use, both in research work and commercial practice, that REED's criticisms and recommendations should be given careful consideration.

This article points out some serious sources of error in the REED method, again demonstrates the reliability of the cold water digestion method, and offers a suggestion as to why, contrary to a tenet held in some quarters, the cold water extraction is effective.

While no attempt will be made to explain the results reported by REED, a variation of 2.5 per cent. sugar between samples from the same beet, which he noted in the cold water digestion method, could be explained by the variation in the percentage of sucrose in the different regions of the same beet. Other investigators have found wide differences in the percentage of sucrose in various regions of the beet. FLODERER and HERKE (3), who worked extensively on this question, reported average percentages of sucrose from different regions of the sugar beet, as follows: 11.51 for crown, 14.76 for shoulder, 17.59 for region of highest sugar, and from 14.40 to 16.01 for lower part of beet. They found in one test that the composited samples of two adjacent pieces from the shoulder region of fifty beets gave respectively

14.76 and 17.29 per cent. sucrose. As these results are averages from many beets, one finds still greater difference in dealing with such samples from individual beets. It is impossible to reconcile these known variations between samples, with the statement by REED (p. 368) in which it is reported that twelve samples from one beet each gave a ten per cent. sugar content value.

REED is undoubtedly correct in his observation that ground beet pulp frequently contains unmacerated bits of tissue consisting of aggregates of unbroken cells. Obviously the common practice of eliminating, before weighing the pulp samples, such pieces of tissue as are large enough to be detected macroscopically amounts to a tacit recognition of the fact that the pulp as used may occasionally contain unmacerated bits of tissue and that fineness of pulp is desirable. It is indeed improbable that any rasp has been or will be designed which will break every cell in the pulp ground. If, in addition to granting this contention, one accepts the opinion expressed by REED that, "any cells which are not broken will not only retain their sugar but will also take up water," errors would reasonably be expected, as he maintains, in the cold water digestion method. The following experiments afford data bearing on the matter.

Experimental

MATERIALS AND METHODS

The sugar-beet sampling machine, which was the pulping device used in this investigation, has been previously described by the writer (6). It will hereafter be referred to as "the machine." In 1924, S. F. SHERWOOD, biochemist, Office of Sugar Plants, Bureau of Plant Industry, United States Department of Agriculture, analyzed by both the hot and cold water digestion methods sugar-beet pulp produced by this machine. He concluded that the pulp produced by this machine was fine enough for cold water digestion. The writer has since confirmed his results by checking the cold water digestion method against the hot alcohol extraction method (1).

The apparatus used in the analyses here reported is calibrated for one-half the normal weight of beet pulp, or 13 instead of 26 grams, and the smaller amount of pulp was consequently used for the samples. The polariscope readings are therefore one-half the actual percentage of sucrose. The samples of pulp except for experiment no. 5 (in which the weights are recorded) and those of sucrose were all weighed to an accuracy of one milligram on an analytical balance. With one exception, which will be noted, 2.6 gram samples of pure sucrose were used, so that the polariscope readings would be of about the same range as those with beet pulp samples.

The clearing of the pulp samples was accomplished by the use of a small amount of basic lead acetate solution (about 2.5 cc. of 1.25 sp. gr., or 30° Baumé, to 13 grams of sugar-beet pulp). In cases where digestion and clearing were effected at the same time, 88.5 cc. of dilute, sp. gr. 1.0073 basic lead acetate were used for each 13-gram sample. It should be noted that the amount of clearing solution used in these experiments was in accordance with standard procedure (1) rather than the concentrated solution (usually used as a stock solution) which REED advises. The clearing solution was measured by a standardized overflow burette, or weighed, and the weights corrected for the density of the solution. In all cases the volumes of the solutions were adjusted before polarization. The polariscope readings were made at 20° C. or the temperature correction applied. The readings for single samples, here recorded to one one-hundredth of a per cent., are the average of three or more separate readings on the same sample. Whenever possible, the help of a second person was obtained to check all readings.

The sugar beets used were fresh and in ideal condition for comparable sampling. Two lots of sucrose were used in the tests: (1) Domino cane sugar, refined by the American Sugar Refining Company, and (2) Saccharose "Difco" Standardized, guaranteed of perfect rotation, containing minimum moisture and ash; chlorides, sulphates, and heavy metals absent. This product is produced by the Digestive Ferments Company. The basic and neutral lead acetates used were of C. P. grade. Distilled water was used for the dilutions.

A twenty-five quart aluminum autoclave was used for these experiments. This autoclave is provided with a standard steam gauge reading in pounds from 1 to 30. It was heated on a coal stove and carefully watched by one person to control the pressure as desired. From 10 to 15 minutes heating was required to bring the pressure to 15 pounds, and 7 to 10 minutes to completely reduce this pressure.

PROCEDURE AND RESULTS

EXPERIMENT NO. 1.—Three samples of pulp were cut, with the machine mentioned, from one sugar beet. These samples were digested in cold water, cleared with basic lead acetate and polarized. The readings[2] were 10.40, 10.43, and 10.71, respectively, or an average of 10.51.

From the same beet, three comparable samples were cut with a knife. These samples were sliced into pieces one-sixteenth of an inch thick and autoclaved with basic lead acetate at 15 pounds pressure for 15 minutes. The readings were 9.24, 9.18, and 9.29, respectively, or an average of 9.24.

[2] To get the percentage of sucrose these readings should be multiplied by 2.

This indicates that treatment of one-sixteenth inch slices of sugar-beet tissue in basic lead acetate with steam at 15 pounds pressure for 15 minutes resulted in lowering the average polariscope reading 1.27, which is equivalent to 2.54 per cent. sucrose.

EXPERIMENT NO. 2.—Two samples were cut with the machine from a uniform beet at the positions indicated by sectors 1-*d* and 2-*d* in figure 1. After cold water digestion these samples read 10.07 and 9.85, or an average of 9.96.
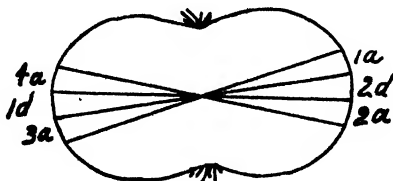
FIG. 1. Cross-section of sampled beet in experiment no. 2 showing position of samples.

Four other samples were cut with a knife from the same beet at positions indicated in the diagram (fig. 1) as 1*a*, 2*a*, 3*a*, and 4*a*. These four samples were each cut into slices one-sixteenth inch in thickness, then treated with basic lead acetate and autoclaved according to Reed's recommendation. The readings from these were 8.50, 8.75, 8.92, and 8.80, respectively, or an average of 8.74.

The REED method in this case gave an average reading showing 2.5 per cent. less sucrose in the samples than was determined by the standard cold water digestion method.

EXPERIMENT NO. 3.—Two composite samples of pulp were cut with the machine from eight beets. After cold water digestion and the usual clearing the extracts read 9.35 and 9.91, or an average of 9.63.

From the same eight beets two comparable composite samples were cut with a knife from the tissue adjoining the machine cuts. After steaming under pressure, as recommended, with basic lead acetate the extracts read 8.35 and 8.90, or an average of 8.63.

Here again the REED method resulted in a very serious error in the sucrose determination, a decrease of 2 per cent. from that determined by the cold water process.

EXPERIMENT NO. 4.—Two composite samples were cut with the machine from five uniform beets. The pulp samples were retained for analysis and the five beets were placed in a closed can and taken to the laboratory of Dr. M. D. THOMAS at the Experiment Station of the American Smelting and Refining Company. Dr. THOMAS then analyzed the beets by the REED method. Following a suggestion from the writer's work on beet tissue

pieces of different sizes, Dr. THOMAS used some samples consisting of thin slices and others of thick slices. The extracts from the thin slices averaged 18.40 per cent. sucrose and those from the thick slices 15.87 per cent. sucrose. The pulp samples, which the writer analyzed, gave an average reading of 10.12, or 20.24 per cent. sucrose.

Again the REED method indicated a distinctly lower percentage of sugar than was shown by the cold water method.

EXPERIMENT NO. 5.—Following a test in which the writer found with the autoclave method that the polariscope readings decreased with an increase in the size of the pieces of beet tissue in the sample, an experiment was planned to clarify this question.

Eight similar samples were cut with a knife from a uniform beet (see fig. 2). Precautions were taken in the sampling with regard to the known facts concerning the distribution of the sugar in sugar-beet roots (3, 7, 8, 10, and 12).
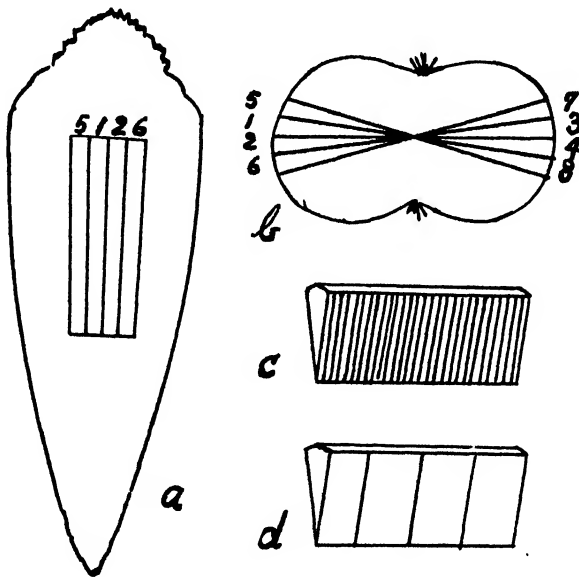
FIG. 2. Method of sampling beet and sectioning the samples.

    a. Shows side view of beet indicating position of samples by number.

    b. Sectional view indicating same.

    c. Method of slicing wedge shaped sample into 1/16 inch slices.

    d. Method of obtaining 3/8 inch slices.

The wedge-shaped pieces of beet tissue were sliced on a glass plate and weighed quickly into flasks, with precaution against evaporation. Samples 1, 3, 5, and 7 were cut into slices not over one-sixteenth of an inch thick. Samples 2, 4, 6, and 8 were cut into three-eighths inch thick slices. The

samples were treated with lead sub-acetate and then steamed under pressure. After the autoclave treatment, samples 3, 5, 4, and 6 were cooled to 20° C., made up to 100-cc. volume, let stand for four hours and then read. The flasks containing samples 1, 7, 2, and 8, which were plugged with cotton before steaming, were allowed to stand seven days and then made up to 100-cc. volume, allowed to stand four hours and then read.

The results of this experiment, as shown in table I, indicate that in the

TABLE I

THE EFFECT OF (1) THE SIZE OF BEET TISSUE PIECES IN THE SAMPLES, (2) THE PERIOD OF EXTRACTION, AND (3) THE AUTOCLAVE EXPOSURE ON THE POLARISCOPE READINGS

SAMPLE NUMBER	WEIGHT OF TISSUE	THICK-NESS OF SECTIONS	EXTRACTION PERIOD	AUTOCLAVING		CORRECTED POLARI-SCOPE READINGS	AVERAGE	SUCROSE
				PRES-SURE	TIME			
	grams*	inch		pounds	minutes			per cent.
1........	13.01	1/16	7 days	10	1	8.45 ⎫		
3........	12.98	1/16	4 hrs.	10	1	8.00 ⎪	8.20	16.40
5........	13.12	1/16	4 hrs.	15	15	8.00 ⎬		
7........	12.90	1/16	7 days	15	15	8.35 ⎭		
2........	13.09	3/8	7 days	10	1	8.25 ⎫		
4........	13.05	3/8	4 hrs.	10	1	7.05 ⎪	7.65	15.30
6........	13.03	3/8	4 hrs.	15	15	7.25 ⎬		
8........	13.15	3/8	7 days	15	15	8.05 ⎭		

* Samples weighed to nearest centigram and readings corrected for 13 grams, weight of sample.

REED method it would be necessary to control the size of the pieces of beet tissue in the samples to obtain a semblance of uniformity of extraction. This is shown by the fact that the finely cut samples 1, 3, 5, and 7 gave an average of 16.40, while the coarser cut samples 2, 4, 6, and 8 gave an average of 15.30 per cent. sucrose. The fact that those four samples read after four hours gave an average reading of 7.58 (15.16 per cent. sucrose) and those four read after seven days an average reading of 8.28 (16.56 per cent. sucrose) indicates that the size of pieces in the tissue samples appreciably affects the time required for diffusion of the sucrose into the solution even if the pieces are autoclaved.

EXPERIMENT NO. 6.—In order to test the effect of autoclaving on ground sugar-beet pulp, the following experiment was conducted. One large sugar beet was sampled several times with the machine in order to obtain a large composite sample of uniform pulp and eliminate the errors in the percentage of sucrose due to irregular distribution of sucrose in the beet. Since some time was required to weigh out this large number of samples and there were dangers of error due to evaporation, the order of weighing out the samples was specially arranged, as shown by the sample numbers

in table II.   The samples to be autoclaved were thus given the advantage and the cold water and alcohol samples were at a disadvantage in being the first and last samples to be weighed out.

These results show that steaming beet pulp in a basic lead acetate solution definitely decreases the polariscope readings.   The results of a more

TABLE II
Effect of Autoclaving on Beet Pulp Treated with Basic Lead Acetate

Sample Number*	Treatment	Polariscope Readings	Average Polariscope Readings for Each Treatment	Equivalent Percentage of Sucrose
1........	Alcohol extraction	9.17	9.16	18.32
29........	"	9.16		
2........	Cold water digestion process	9.21		
3........	"	9.20		
4........	"	9.15		
5........	"	9.20		
6........	"	9.20	9.19	18.38
25........	"	9.22		
26........	"	9.20		
27........	"	9.17		
28........	"	9.20		
7........	Autoclaved at 15 pounds for 15 minutes with basic lead acetate	9.13		
8........	"	9.11		
9........	"	8.95		
10........	"	9.00		
11........	"	9.07	9.04	18.08
12........	"	8.95		
13........	"	9.00		
14........	"	9.00		
15........	"	9.14		
16........	Autoclaved at 15 pounds for 15 minutes with distilled water	9.20		
17........	"	9.17		
18........	"	9.25		
19........	"	9.10		
20........	"	9.20	9.17	18.34
21........	"	9.20		
22........	"	9.20		
23........	"	9.13		
24........	"	9.10		

* Numerical order indicates order in which samples were weighed.

extensive experiment confirmed this point.   It is worthy of note, too, that the samples steamed under pressure with basic lead acetate were shown by the MUNSON and WALKER method to have 50 per cent. more direct reducing sugars than did those given the cold water digestion process.   The extraction by alcohol was proved to be complete by the alpha naphthol test.

EXPERIMENT NO. 7.—The purpose of this test was to study the effect of different exposures to steam pressure on finely ground sugar-beet pulp in distilled water. Samples were weighed out from a composite sample of machine-cut pulp, autoclaved with distilled water, cleared, made up to volume and polarized. The results are shown in table III.

These results indicate that prolonged exposures of sugar-beet pulp to steam under pressure, even when in distilled water, appreciably decreases the amount of sucrose, but that short exposures at ten pounds, or even twenty-seven pounds pressure does not cause an appreciable decrease.

EXPERIMENT NO. 8.—The following experiment was planned to determine the effect of autoclaving chemically pure sucrose with each of the following solutions: Basic lead acetate, 1.0073 sp. gr.; neutral lead acetate, 0.5 cc. in 100-cc. volume of solution; and distilled water.

TABLE III

EFFECT OF DIFFERENT LENGTHS OF EXPOSURE TO STEAM UNDER PRESSURE ON SUGAR-BEET PULP IN DISTILLED WATER

SAMPLE NUMBER	EXPOSURE		POLARISCOPE READINGS
	TIME	PRESSURE	
	minutes	pounds	
1	45	15	7.34
2	45	15	7.37
3	1	10	7.59
4	1	10	7.56
5	1	27	7.52
6	1	27	7.55
7 Check*........	—	—	7.55
8 Check*......	—	—	7.56

* Cold water digestion.

The results (table IV) indicate that pure sucrose in distilled water is slightly decomposed by steam at 15 pounds for 15 minutes and more so by prolonged exposure. They also show that in basic lead acetate solution sucrose is even more markedly decomposed by steam under pressure. Samples 16, 17, 18, and 19 were acid to litmus after autoclaving. Samples 10 and 11 reduced an alkaline copper salt solution at 70° C. in 5 minutes, while samples 16 and 17 showed marked reduction within one minute at room temperature. These results show that even pure sucrose can not be safely autoclaved with either of the lead solutions at 15 pounds for 15 minutes.

TABLE IV

THE EFFECT ON PURE SUCROSE OF AUTOCLAVING IT IN DISTILLED WATER WITH VARIED
EXPOSURES AND ALSO IN BASIC AND NEUTRAL LEAD ACETATES

SAMPLE NUMBER	SOLUTION	STEAMING		POLARISCOPE READINGS
		TIME	PRESSURE	
		minutes	*pounds*	
1 Check				10.00
2 Check				9.99
3 Check				10.00
4 Check				9.99
5 Check				10.00
6	Distilled water	15	15	9.95
7	"	15	15	9.95
8	"	15	15	10.00
9	"	15	15	9.98
10	"	120	15	9.85
11	"	120	15	9.60
12	Basic lead acetate	15	15	8.90
13	"	15	15	9.28
14	"	15	15	9.15
15	"	15	15	8.85
16	"	120	15	8.10
17	"	120	15	8.65
18	Neutral lead acetate	15	15	9.67
19	"	15	15	9.73

EXPERIMENT NO. 9.—The preceding experiment was repeated using one-half normal weights (13.000 grams) of pure sucrose per 100 cc. solution, and treated, as shown in table V.

These results definitely confirm the results of experiments 6 and 8, and show that the polarization of sucrose is reduced by autoclaving at 15 pounds for 15 minutes, and strikingly so when basic lead acetate is present.

EXPERIMENT NO. 10.—The increased acidity of sugar-beet pulp due to autoclaving was demonstrated by digesting 13.000-gram samples of beet pulp in 88.5 cc. of dilute basic lead acetate, or water, and following the standard beet-sugar factory method for titration of acid solutions. In 100 cc. of filtrate from beet pulp autoclaved at 15 pounds for 15 minutes with basic lead acetate, the acidity corresponds to 0.058 gram CaO, while that for the check solution not autoclaved was only 0.044 gram CaO. In other words, 14 cc. of N/28 alkali were required to neutralize the increased accumulation of acid in the beet pulp due to autoclaving. The increased acidity of sugar-beet pulp autoclaved in distilled water at 15 pounds for 15 minutes amounted to 0.010 gram CaO, or 10 cc. of N/28 alkali. These

TABLE V

THE EFFECT OF AUTOCLAVING ON SUCROSE DISSOLVED IN DISTILLED WATER AND IN DILUTE BASIC LEAD ACETATE SOLUTION

SAMPLE NUMBER	WEIGHT SUCROSE	VOLUME OF SOLUTION	TREATMENT	POLARISCOPE READINGS AT 20° C.
	gm.	*cc.*		
1	13.000	100	Distilled water (check)	49.80
2	13.000	100	Autoclaved at 15 pounds for 15 minutes with distilled water	49.55
3	32.500	250	Distilled water (check)	49.85
4	32.500	250	Autoclaved at 15 pounds for 15 minutes with distilled water	49.55
5	32.500	250	Autoclaved at 15 pounds for 15 minutes with dilute basic lead acetate solution	47.65*

* This sample was so badly discolored that a tenfold dilution was required before reading.

results furnish an explanation of the reduced polarization of sucrose and of sugar-beet pulp after being autoclaved.

EXPERIMENT NO. 11.—This experiment was planned to determine what, if any, percentage of the sugar-beet cells in finely ground pulp retained their sucrose after the cold water extraction. A composite sample was cut by the machine from three sugar beets. Two 13-gram samples, cold water digested, read 9.45 and 9.43, or an average of 18.88 per cent. sucrose. Four other 13-gram samples were weighed out and washed repeatedly by pouring cold water over the pulp in funnels. After 24 hours the wash waters gave a negative alpha-naphthol reaction showing complete absence of sucrose. This pulp was examined under a microscope and found to be made up of aggregate of cells three or more layers thick. The washed samples were then transferred to 100-cc. sugar flasks and digested 30 minutes at 75° to 80° C. Each of these hot water extractions read zero in the polariscope and gave negative alpha-naphthol reactions. This shows that the hot water had failed to extract additional sucrose, and therefore that the cold water had extracted the sucrose completely.

As some might question the effect of the long period (24 hours) of cold water washing, the experiment was repeated. The beet pulp used tested 18.15 per cent. sucrose. Two hours were required to completely wash the three samples with cold water under ordinary pressure. Between 500 and 700 cc. of water were used for each 13-gram sample. The samples were

transferred to 100-cc. flasks and hot water digested. Each filtrate gave zero polariscope reading and all but one a negative alpha-naphthol reaction. The reaction in this one case was very faint. This confirms in general the previous test. These results show that cold water digestion is effective with finely ground pulp.

EXPERIMENT NO. 12.—In order to check the cold water digestion method with the freezing method, a composite sample of uniform pulp was cut by the machine from one beet. Three samples of this pulp after digesting with cold basic lead acetate read 8.52, 8.53, and 8.52, respectively. Five samples of this pulp were frozen with solid carbon dioxide for two hours. These samples after clearing read 8.53, 8.52, 8.54, 8.52, and 8.51, respectively. These results show that for finely ground pulp the cold water digestion method is as accurate as the freezing method.

Four comparable samples were cut with a knife from this same beet that furnished the pulp samples. The first two samples were sliced to a thickness of one-fourth inch or less, frozen as stated above, and read as 1.30 and 1.00, while the other two samples sliced to a thickness of one-sixteenth inch or less read 2.30 and 3.52, respectively. One and one-half hours were allowed for clearing and diffusion before reading. These results confirm the results of experiment 5, and show that the freezing method, as well as the autoclave method, requires a finely divided pulp. It is noteworthy that the results obtained here by the freezing method are not in harmony with the uniform results which REED obtained from 15 samples (*loc. cit.*, p. 369).

Discussion

Regarding the cold water digestion of sugar-beet pulp, the case is simple. If the pulp is ground fine enough to permit of complete extraction, no point is to be gained in autoclaving since heating under pressure is extremely likely to cause changes which materially affect the specific rotatory power. If the pulp is not fine enough for cold water extraction, the hot water or alcohol method should be used.

While some low speed rasps and food choppers cut too coarse a pulp for cold water extraction, this is not the case with the disc machine devised by the writer. The disc, which is of special design for cutting a fine pulp, runs at 1725 rpm. and cuts a pulp fine enough for cold water extraction. Extensive earlier experiments of the writer as well as those of other workers (2, 11), show that the cold water digestion method is, with due precautions, accurate to within one-tenth of one per cent. It is a rapid method which affords a degree of accuracy sufficient for the critical testing of beets for genetical purposes as well as for various phases of physiological research.

In general commercial practice the cold water digestion method is widely used in connection with beet-sugar factory operation and the settlement of grower contracts involving the percentage of sucrose in the beets. Here also a reasonable degree of accuracy is necessary, and, naturally, control analyses by hot water digestion are frequently run. All this procedure is clearly contradictory to REED's data and consequently to his conclusions.

The basic assumption on which REED's work was done is that the unbroken cells would not allow the sucrose in them to escape. From the experiments here reported, it appears that the plasma membranes of the unbroken cells of a fine pulp are rendered permeable to sucrose. Experiment no. 11 shows that sucrose under cold water extraction diffuses readily from finely ground pulp. Presumably this is due to certain phenomena associated with the stimulus of wounding (5). This suggestion regarding the probable explanation of the effectiveness of cold water extraction is worthy of special attention.

Regarding the second major point of interest—the autoclave method—it will be noted that one very important point was overlooked. Even though sucrose is relatively stable in alkaline solution (4), in the writer's tests pure sucrose was not stable when autoclaved at 15 pounds for 15 minutes with either distilled water, neutral lead acetate, or basic lead acetate solutions. This is in harmony with other reports, e.g. BROWN (2) quotes HERZFELD to the effect that sucrose dissolved in water is decomposed by heating. Further, the results here reported show that by exposure to high temperature and pressure, the solution containing basic lead acetate and sucrose was completely neutralized and direct reducing sugars were formed. It is a regular part of the commercial refining process to neutralize the acids in the extracted beet juice by means of lime in order to prevent losses of sucrose. The results of the writer's experiments along this line are quite in harmony with these long established facts and practices. REED says that the autoclave method yields a higher sugar reading than does the cold water digestion process and that it does not affect refined sugar when this is added to samples with lead sub-acetate before heating. The writer has shown that the autoclave method consistently and seriously decreases the sucrose reading of beet juice; that the method results in decomposition of pure sucrose, and that the polariscope reading of even pure sucrose in distilled water or lead acetate solutions is lowered by the autoclaving REED recommends for beet tissue samples.

One other point of minor interest should be mentioned. REED states that "chunk" samples are as satisfactory as pulp with the autoclave method. The writer's results show (experiments 4 and 5) that complete diffusion from such "chunks" requires days (as opposed to minutes) for

extraction if the size of the pieces in the samples are of the type REED evidently proposes for use. These statements are equally true when applied to the freezing process (experiment 12). If finely ground pulp is used, the freezing method appears to have fewer objectional features than the autoclave method, since no reduction was noted in the sucrose reading of frozen pulp. However, no good reason for the recommendation of either is evident. The practice formerly followed in the writer's work, in taking pulp samples, has been to sharpen the disc of the machine each year, but in the 1929 tests it was used the second season without sharpening. As a result, the pulp used in these experiments has not been quite so fine as the pulp used in previous investigations. The pulp cut at this time contained many shreds which have not appeared in the pulp from the newly sharpened disc. The fact that under these conditions the machine still cuts a pulp sufficiently fine for cold water digestion, very decidedly strengthens the results and arguments here presented.

An attempt to discard a long established method without an abundance of carefully confirmed supporting evidence is very regrettable. In this instance not only will it cause confusion in investigational work but it is likely also to make trouble in beet-sugar factory operations. Unless promptly corrected, the mistaken criticism of the cold water digestion method is likely to arouse questions in the settlement of grower contracts where accurate determination of the percentage of sugar in the beets is presupposed.

Acknowledgments are due to Dr. EUBANKS CARSNER for helpful suggestions and criticisms.

Summary

1. It was experimentally demonstrated that the cold water digestion of finely ground sugar-beet pulp gave higher and more consistent polariscope readings than did the comparable samples autoclaved, as recommended by REED.

2. Evidence was secured that the time required for complete extraction of sugar from autoclaved or frozen beet tissue varied directly with the size of the tissue pieces in the sample.

3. Autoclaving at 15 pounds for 15 minutes with either basic or neutral lead acetate reduced the polariscope readings of sugar-beet pulp and of pure sucrose. This polariscope reading decreased as the time of autoclaving was increased.

4. Pure sucrose was measurably decomposed by autoclaving at 15 pounds for 15 minutes with basic lead acetate.

5. The polarization of pure sucrose dissolved in distilled water was also lowered by autoclaving.

6. The results obtained show that the cold water digestion method for the determination of sucrose in sugar-beet tissue is superior to autoclave digestion, and that the cold water extraction method can be depended upon when finely ground pulp is used. It is suggested that the extraction of sucrose by cold water from unbroken living cells may be due to changes in permeability resulting from wounding of the tissue.

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LITERATURE CITED

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis, p. 205. 2nd ed. Washington, D. C. 1925.
2. BROWNE, C. A. Handbook of sugar analysis. First ed. Wiley & Sons, New York. 1912.
3. FLODERER, A., and HERKE, A. Über die Verteilung des Zuckers sowie der Nichtzuckerstoffe in der Zuckerrübe. Öster-Ungar. Zeit. Zuckerind. und Landw. 40: 385–397. 1911.
4. MATHEWS, A. P. Physiological chemistry, Second ed. William Wood & Co. New York. 1916.
5. MOLISCH, H. Über localen Blutungsdruck und seine Ursachen. Bot. Zeitg. 60: 45–63. 1902.
6. PACK, D. A. A new disc sampling machine for mother beets. (Abstract) Sugar 26: 97. (A complete description is available in the U. S. Department of Agriculture Library). 1924.
7. PEKLO, J. Histochemisches uber die Lokalisation der Saccharose in der Zuckerrübe. Öster-Ungar. Zeit. Zuckerind. und Landw. 37: 153–174. 1908.
8. PLOT, J. Der Zuckergehalt der Rüben nach äusseren Merkmalen der Wurzel; Verteilung des Zuckers nach der Lange und Breite. Blatt f. Zuckerrübenbau. 5: 357–361. 1889.
9. REED, ERNEST. Testing the sugar content of beets for genetical purposes. Plant Physiol. 4: 367–371. 1929.
10. SCHUBART, P. Zur Verteilung des Zuckers in der Rübe. Centralbl. Zuckerind. 14: 994–996. 1906.
11. SHERWOOD, S. F. Determination of sucrose in seed beets. Sugar 23: 299–300. 1921.
12. URBAN, J. Topographie des Zuckers und Nichtzuckers in der Zuckerrübenwurzel. Zeit. Zuckerind. in Böhmen 32: 17–24. 1907.

ESTIMATION OF NITRATE NITROGEN IN PLANT JUICE: A STUDY OF THE EXPRESSION AND CLARIFICATION OF THE JUICE[1,2]

DONALD E. FREAR

(WITH ONE FIGURE)

Introduction

Since the publication by GILBERT (3) of the colorimetric method for the determination of nitrate nitrogen, phosphate, and potash in plant juices, several workers have presented criticisms and modifications of this method as applied to nitrates. These include particularly HOLTZ and LARSON (7) and EMMERT (2). Considerable work has been done in this laboratory on this particular determination and the purpose of this paper is to present the modifications which have evolved here during the past four years.

The GILBERT method is essentially as follows: The fresh plant tissue is ground and the juice expressed through fine mesh cloth. This juice is decolorized with carbon black and finally clarified by the addition of solutions of $AgSO_4$, $CuSO_4$, and a mixture of solid $Ca(OH)_2$ and $MgCO_3$ as recommended by HARPER (5). It was originally the plan to determine nitrate nitrogen, phosphorus, and potash on aliquots of the original juice. As the phosphate determination does not permit the use of alkaline reagents for clarification, the treatment with carbon black was extremely important. Later it was believed that heating would coagulate much of the colloidal material, allowing its separation with the carbon black on the filter. This was adopted shortly after publication of the original method, but has never been described in the literature.

HOLTZ and LARSON (7) have published a criticism of the recovery of nitrate nitrogen by the GILBERT method, stating that, using the procedure as published, they were only able to recover 40–45 per cent. of the nitrate added to extracts of wheat plants. This extremely low recovery they attribute to the use of $MgCO_3$ in the final clarification, which prolonged the final evaporation. These workers have suggested a modified procedure

[1] Contribution no. 390 of the Rhode Island Agricultural Experiment Station.

[2] The product collected when plant tissues have been subjected to pressure has been variously termed sap, juice, plant solution, tissue fluid, expressed plant tissue fluid, etc. In order to reduce this confusion of names the author suggests that the word "juice" be used as defined in the Oxford Dictionary; "The watery or liquid part of vegetables or fruits which can be expressed or extracted." The New Webster's International Dictionary defines juice: "The extractable fluid contents of plant cells or plant structures, consisting of water holding sugars or other substances in solution."

in which the AgSO$_4$ and CuSO$_4$ solutions, the carbon black, and the Ca(OH)$_2$ are added at the same time, and filtered without heating. HOLTZ and LARSON further report that this method gives them 90–100 per cent. recovery of added nitrate nitrogen, but their data show no higher results on the original sample of extract than those obtained by the GILBERT method.

The use of carbon black has been criticized by EMMERT (2), who cites the theoretical possibility of reduction of nitrates in the solution in the presence of the carbon black. In addition, he mentions the difficulty in obtaining suitable carbon black, and the possibility of relatively great adsorption and occlusion with some brands.

The GILBERT method as it was originally described gave results for nitrate nitrogen, phosphorus, and potash in the juice of plants that correlated with the amounts of these elements applied to the substrate upon which the plants were growing, and with crop yields. The method has then fulfilled the requirements which were imposed upon it, and it still is to be considered sufficiently accurate to indicate any large differences which may exist in crop juices. The modified method presented in this paper includes several refinements in technic that have improved the accuracy of the method so that it more nearly measures the true quantity of nitrate nitrogen in the plant.

Modifications in the method

COMPARISON OF METHODS FOR OBTAINING THE JUICE

Considerable difficulty was experienced in obtaining sufficient juice by the grinding and straining procedure, especially from small samples. There is some question whether the juice so obtained is a representative aliquot of the juice present in the plant, and it was felt that a more suitable method for securing samples of juice was desirable. Several workers (GORTNER and HARRIS (4), KORSTIAN (8), NEWTON, BROWN and MARTIN (12), etc.), have used an ice-salt bath to freeze the plant tissue. This process was used in this laboratory with considerable success, but in some cases the plant tissue was extremely resistant to freezing at the temperature resulting (-10 to $-15°$ C.). Later, following the method of HARVEY (6), MEYER (11), LEWIS and TUTTLE (9), and others, solid carbon dioxide was used as a freezing medium with greater success. The temperature was so low that there was no question of the thoroughness of the freezing when the tissue was placed in contact with the solid CO$_2$. There has recently been placed on the market a machine which manufactures small cakes of the solid CO$_2$ from the compressed gas in drums. This eliminates purchasing large quantities of the solid CO$_2$, and the cost of freezing individual samples is low.

It is unlikely that the substitution of solid CO_2 for the ice-salt bath would affect the recovery of nitrate nitrogen from the resulting juice, since MEYER (11) cites evidence that there is little difference in osmotic pressure between samples of juice expressed after freezing with liquid air, solid CO_2, and ice-salt mixture. No direct comparison has been attempted in this laboratory.

After allowing the sample of plant tissue to remain in contact with the solid CO_2 for four hours or more, it is thawed, and pressed with mechanical pressure. An especially designed press cage has been used with good results in a hydraulic press maintaining 1000 pounds per square inch pressure on the tissue.

The cage used was designed to give the greatest ease of cleaning together with the lowest cost of manufacture. Three parts make up the entire assembly: The *sleeve, A,* is a six inch length of cold drawn seamless steel tubing with an outside diameter of three inches, and one-eighth inch walls. The *base plate, B,* is machined from a three-inch cast iron disc three-fourths inch thick to a diameter one sixty-fourth inch less than the inside diameter of the sleeve, leaving a one-quarter inch flange to allow the plate to extend only one-half inch into the sleeve. The *plunger, C,* is machined from a three inch bar of cast iron, six and one-half inches long, leaving a half-inch flange at one end to facilitate removal. This plunger is machined to 0.001 inch of the inside diameter of the sleeve. (See figure 1).

The dimensions given may be varied to suit the individual requirements: A cage made from one-inch tubing two and one-half inches long is very convenient for small samples. These cages can be readily taken apart and cleaned, a convenient feature when a large number of determinations are to be made.

There is a small hydraulic press on the market which is convenient, satisfactory, and relatively inexpensive, and which is designed for the expression of juices and oils. The juice as it is expressed from the tissue flows from the lower end of the cage and into a gutter in the press plate of the press, from which it discharges into a beaker.

The physical character of the juice from the above procedure is markedly different from that obtained when the tissue is ground. The juice from frozen tissue is practically free from cell débris and contains only small quantities of protoplasm, whereas the juice from ground tissue contained much colloidal material.

CARBON BLACK AS A DECOLORIZING AGENT

The treatment with carbon black was necessary in the original preparation of the juice, since, as has been noted above, two other determina-
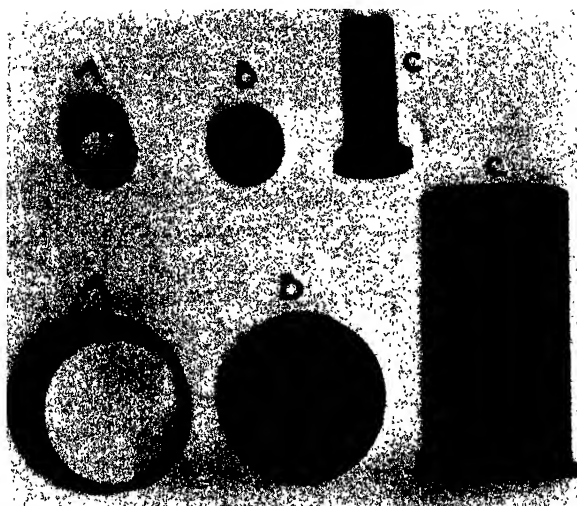
FIG. 1. Press cages used in the expression of plant juices: *A*, sleeve; *B*, base plate; *C*, plunger.

tions were to be made on aliquots of the sample. Since this work is directly concerned only with the determination of nitrate nitrogen, it was found that the reagents recommended by HARPER (5), with slight modifications, removed all of the coloring matter without the preliminary carbon black decolorization. In addition to this, there is considerable possibility

TABLE I

RECOVERY OF NITRATE NITROGEN FROM SYNTHETIC SOLUTION WHEN DIFFERENT BRANDS OF
CARBON BLACK WERE USED. TWENTY-FIVE CC. OF A SOLUTION CON-
TAINING 100 PPM. NITRATE NITROGEN PLUS 2.5 GM.
OF CARBON BLACK

BRAND	NITRATE NITROGEN RECOVERED
	ppm.
None	100
A	95
B	105
C	109
D	99
E	14

that the carbon black will adsorb or occlude some of the nitrate nitrogen present in solution. Some carbon blacks, on the other hand, contain nitrates and perhaps free nitric acid. A trial of five brands of carbon blacks is shown in table I.

The fact that the determination could be made on juices without carbon black made possible a study of the amounts actually removed from beet juices by the black itself (table II).

TABLE II

RECOVERY OF NITRATE NITROGEN FROM BEET JUICES WITH AND WITHOUT CARBON BLACK
TWENTY-FIVE CC. OF JUICE PLUS 2.5 GM. OF CARBON BLACK

SAMPLE NUMBER	PLUS CARBON BLACK	NO CARBON BLACK
1	563	667
2	526	602
3	641	735
4	379	757

These figures indicate that carbon black introduces the possibility of serious error. This agrees with the findings of EMERSON (1) and LIPMAN and SHARP (10), who worked with carbon black and animal charcoal. No attempt has been made to determine the way in which the carbon black operates to remove nitrate nitrogen.

EMMERT (2) states that there is the possibility of serious error through the continuation of reduction of nitrate nitrogen in the extract after the addition of carbon black. To the author the possibility of this taking place seems very slight. While there is no direct evidence to be offered to prove that no reduction takes place, table III gives several results which indicate that change in the quantity of nitrate nitrogen recovered is small

TABLE III

NITRATE NITROGEN RECOVERED BY GILBERT METHOD FROM LEAVES AFTER STANDING

CROP (LEAVES)	NITRATE NITROGEN DETERMINED ONE-HALF HOUR AFTER PICKING	TEMPERATURE OF ROOM	NITRATE NITROGEN DETERMINED IN LATER DETERMINATION	TIME ELAPSING BETWEEN DETERMINATIONS
	ppm.	*degrees*	*ppm.*	*hours*
Beet (midribs removed) ...	649	25	641	7
Beet (midribs removed) ...	58	25	70	5
Cabbage (midribs removed)	285	27	273	6.5

even though the juice is allowed to stand for a considerable time. These results were obtained by halving each leaf immediately after collection,

determining nitrate nitrogen in one-half at once and allowing the other half to remain at the temperature shown for the time indicated.

The juice is in contact with the carbon black for a very short time, probably not over five minutes in most cases. While the theoretical possibility that the reduction of nitrates may continue, due to catalytic action of the carbon black or plant reducing substances is acknowledged, it has been the experience of the author that this is so slight under normal conditions that it may be disregarded. It is to be noted that EMMERT secured considerable reduction only when he used metallic zinc with NaOH, a much more powerful reducing agent than normally occurs in plant tissues.

The use of carbon black is not advised without an extremely careful investigation of the effect of the carbon black chosen on nitrates in solution. In this procedure the use of carbon black is eliminated.

CLARIFICATION REAGENTS

The quantities of reagents used in the final clarification of the juice and removal of the chlorides have been changed considerably from the amounts recommended by HARPER in his original procedure. The quantity of saturated $AgSO_4$ solution used to precipitate chlorides has been reduced from 10 cc. to 5 cc., since it has been observed that the latter amount is sufficient to precipitate the chlorides present in normal juices. EMMERT recommends that the treatment with $AgSO_4$ be omitted when the chlorine content is below 20 ppm. It has been noted in this laboratory that most of the juices under examination contain more than the above designated quantity. Any considerable excess of silver, however, leaves a brown or black mirror when the solution is evaporated preliminary to the treatment with phenoldisulphonic acid.

GILBERT has recommended that 0.5 cc. of N $CuSO_4$ be used to precipitate the proteins present in the juice. This quantity is sufficient in most instances, but the use of 1.0 cc. of N $CuSO_4$ is more likely to satisfy all conditions. Table IV shows the results obtained when clarification reagents are omitted or substituted in various ways.

These data substantiate the findings of several workers, that some basic material is necessary to precipitate the silver and copper as the hydroxides. Undoubtedly the greater part of the clarification is brought about by these hydroxides, and it is essential to have them in the state of division which will give the maximum adsorption of coloring material. It is evident that $MgCO_3$ is not suited to be used alone in this connection, as there remained a dark residue after evaporation. Sodium hydroxide gave a finely divided precipitate of colloidal hydroxides which could not be removed by filtering. The use of $Ca(OH)_2$ proved to be as efficacious as the mixture with

TABLE IV

INFLUENCE OF AMOUNT AND KIND OF CLARIFICATION REAGENTS ON THE PHYSICAL PROPER-
TIES OF THE RESIDUE FROM EVAPORATION OF A FILTERED ALIQUOT AND THE
FINAL COLOR DEVELOPED BY PHENOLDISULPHONIC ACID REAGENT

TREATMENT	COLOR OF RESIDUE BEFORE ADDITION OF PHENOL-DISULPHONIC ACID	COLOR OF FINAL SOLUTION
2 cc. of juice made to 100 cc. final volume including:		
1. 5 cc. saturated AgSO₄: 0.5 cc. N CuSO₄ 0.2 gm. Ca(OH)₂ + 0.5 gm. MgCO₃	White	Yellow
2. 5 cc. saturated AgSO₄	Black	Black
3. 5 cc. saturated AgSO₄ + 1.0 cc. N CuSO₄	Green	Black
4. 5 cc. saturated AgSO₄ + 1.0 cc. N CuSO₄ + 0.2 gm. Ca(OH)₂	White	Yellow
5. 5 cc. saturated AgSO₄ + 1.0 cc. N CuSO₄ + 0.4 gm. MgCO₃	Brown	Black
6. 5 cc. saturated AgSO₄ + 0.5 cc. N CuSO₄ + 0.2 gm. Ca(OH)₂	White	Yellow
7. 5 cc. saturated AgSO₄ + 0.5 cc. N CuSO₄ + 1 cc. 20 per cent. NaOH	Black	Black
8. Same as 7, but with 2 cc. 20 per cent. NaOH	Black	Black
9. Same as 7, but with 5 cc. 20 per cent. NaOH	Black	Black

$MgCO_3$. This substantiates the work of HOLTZ and LARSON, who found that the $Ca(OH)_2$ alone, was sufficient in the clarification. However, it was not found that the addition of $MgCO_3$ (although it reduced the solubility of the basic precipitant) influenced the time of evaporation, as these workers indicate.

The use of $Ca(OH)_2$ alone, to replace the mixture of $Ca(OH)_2$ and $MgCO_3$ gave slightly higher results, although the magnitude of this increase appears to be within the experimental error of the method (table V).

TABLE V

NITRATE NITROGEN RECOVERED, USING 5 CC. SATURATED AgSO₄ AND 1.0 CC. N CuSO₄ WITH
0.2 GM. Ca(OH)₂ ALONE AND IN COMBINATION WITH 0.5 GM. MgCO₃

| CROP (LEAVES) | NITRATE NITROGEN | | | DIFFERENCE |
	REAGENTS + CA(OH)₂ + MgCO₃	REAGENTS + CA(OH)₂	DIFFERENCE	
	ppm.	ppm.	ppm.	per cent.
Tomato	371	386	+ 15	+ 4.0
"	379	390	+ 11	+ 2.9
Spinach	140	147	+ 7	+ 5.0
"	144	148	+ 4	+ 2.7

From the results it is recommended that 0.2 gram $Ca(OH)_2$ be used in place of the mixture of $Ca(OH)_2$ and $MgCO_3$.

The original procedure recommended by GILBERT directs that after the addition of the clearing reagents the solution shall be heated. It seemed possible that the heating would alter the adsorption equilibrium which exists between the colloidal precipitate and the nitrates in solution. When the solution containing the clearing reagents was allowed to stand for one hour at room temperature clarification was as complete as when heated. A series of determinations was made to compare recovery of nitrate by the two treatments (table VI).

TABLE VI

NITRATE NITROGEN RECOVERED FROM JUICES. HEATING AFTER THE ADDITION OF CLEARING REAGENTS COMPARED WITH ALLOWING TO STAND AT ROOM TEMPERATURE

CROPS (LEAVES)	NITRATE NITROGEN			DIFFERENCE
	HEAT	NO HEAT	DIFFERENCE	
	ppm.	ppm.	ppm.	per cent.
	227	231	+ 4	+ 1.8
	259	333	+ 74	+ 28.6
Beet (midribs removed	323	398	+ 75	+ 23.2
	83	92	+ 9	+ 10.8
	270	300	+ 30	+ 11.1
	229	291	+ 62	+ 27.1
Cabbage (midribs removed)	377	321	− 56	− 14.8
	371	411	+ 40	+ 10.8
	286	244	− 42	− 14.7
Celery	360	360	0	0.0
	85	103	+ 18	+ 21.2
Mangels (midribs removed)	94	85	− 9	− 9.6
Spinach	125	140	+ 15	+ 12.0
	138	144	+ 6	+ 4.3
	296	285	− 11	− 3.7
	379	393	+ 14	+ 3.7
	76	76	0	0.0
	92	90	− 2	− 2.2
Tomato	83	111	+ 28	+ 33.7
	138	118	− 20	− 14.5
	70	74	+ 4	+ 5.7
	28	36	+ 8	+ 28.6
	353	366	+ 13	+ 3.7
	185	200	+ 15	+ 8.2
Potassium nitrate solutions	185	199	+ 14	+ 7.6
	189	195	+ 6	+ 3.2
	192	183	− 9	− 4.7
Averages.			+ 10.6	+ 6.7

The data given in this table indicate that, while the variation in the different individual determinations is great, by far the greater number of results show a higher value for nitrates when no heat is used in the clarification. Since the heating and cooling of the sample consumed considerable time, and the results indicate a less complete recovery of nitrates, it was decided to dispense with the heating.

At times a white precipitate will be formed when the solution containing the phenoldisulphonic acid is neutralized with NaOH. This may be removed, as can a brown precipitate which is rarely encountered at this point by allowing the precipitates to flocculate and filtering.

METHOD RECOMMENDED

Place in a cheesecloth bag a sample of fresh plant tissue sufficient to yield a minimum of 10 cc. of juice, and freeze thoroughly for at least two hours. Either an ice-salt bath or solid CO_2 may be used, although the latter is preferable. Remove from refrigerating medium, allow to thaw, and press at once in any apparatus which will give sufficient mechanical pressure. It is best to have arrangements for duplication of this pressure on comparative samples. A hydraulic press with pressure gauge is the most satisfactory equipment. Collect the expressed juice, which should be free from cell residues, and pipette an aliquot (usually 2 cc.) into a volumetric flask (100-cc.).

To the juice in the flask, add about 20 cc. of nitrate-free distilled water; 5 cc. saturated $AgSO_4$ solution, 1 cc. N $CuSO_4$ solution, and 0.2 gram finely divided C. P. $Ca(OH)_2$, shaking after each addition. Shake thoroughly, make to volume with nitrate-free distilled water and filter after standing at least one hour. Discard first portion of the filtrate. Take a suitable aliquot (10 to 50-cc.) of the clear, colorless filtrate, evaporate to dryness on a water bath without overheating, and determine nitrate nitrogen by the phenoldisulphonic acid method, using NaOH to neutralize the acid according to HARPER (5). If a precipitate forms at this point, allow to flocculate and filter.

Recovery of added nitrates by the modified method

The recovery of nitrate nitrogen by the above method was determined by adding a known amount of KNO_3 to the juices of different crops. Measured amounts of a standardized solution of KNO_3 were placed in evaporating dishes, evaporated to dryness, and the residue dissolved in a definite quantity of juice. This procedure obviated any change in the adsorption relation of the juice caused by dilution (table VII).

It is to be noted that in only three cases does the recovery exceed 100 per cent. The lowest recovery in the data is 78.1 per cent. and the average

TABLE VII

RECOVERY BY MODIFIED METHOD OF NITRATE NITROGEN ADDED TO PLANT JUICES

CROPS (LEAVES)	NITRATE NITROGEN			RECOVERY
	ORIGINALLY IN JUICE	ADDED TO JUICE	RECOVERED IN JUICE	
	ppm.	ppm.	ppm.	per cent.
Beet (midribs removed)........	163	400	488	86.7
	163	1400	1538	98.4
	227	50	240	86.6
	231	100	333	100.6
	231	200	398	92.3
Cabbage (midribs removed)	229	100	377	114.6
	229	200	371	86.5
	291	100	321	82.1
	291	200	411	83.7
	230	50	229	81.8
	230	100	286	86.7
	230	200	360	83.7
Mangels (midribs removed)	42	50	75	81.5
Spinach	30	400	353	82.1
	30	1000	822	79.8
	30	2000	1935	95.3
Tomatoes	76	25	90	89.1
	76	50	111	88.1
	76	75	118	78.1
	214	50	233	88.3
	214	100	296	94.3
	214	200	379	91.5
	270	100	390	105.4
	270	200	448	95.3
	270	250	513	98.7
Average				90.05

of 25 results is 90.05 per cent. This average recovery equals that of HOLTZ and LARSON (7) in one of their two trials.

Comparison of the two methods

When the GILBERT method was compared with the procedure outlined above the differences found were not consistent but were too great in some instances to be disregarded (table VIII).

The discrepancies may be attributed in part to the fact that the grinding of the sample did not accomplish a complete maceration of all the plant cells, and that probably in many cases the juice secured was not a representative aliquot of the juice of the plant as a whole. As has been mentioned previously, many workers have found that the juice obtained

TABLE VIII
NITRATE NITROGEN DETERMINED BY THE GILBERT METHOD COMPARED WITH THAT BY THE RECOMMENDED MODIFICATIONS

CROP (LEAVES)	NITRATE NITROGEN			DIFFERENCE
	GILBERT METHOD	MODIFIED METHOD	DIFFERENCE	
	ppm.	ppm.	ppm.	per cent.
Beet (midribs removed)	690	667	− 23	− 3.3
	641	757	+116	+18.1
	116	92	− 24	−20.7
	95	103	+ 8	+ 8.4
	135	95	− 40	−29.6
	148	112	− 36	−24.3
Cabbage (midribs removed)	67	105	+ 38	+57.0
Celery	183	183	0	0.0
Tomato	628	600	− 28	− 4.5
	637	607	− 30	− 4.7

by freezing and pressing the tissue has considerably different physical properties from that secured by grinding the tissue and extracting the juice under variable pressure.

In order to demonstrate that the amount of nitrate nitrogen found in the juice obtained by the grinding process was not comparable with that in the juice obtained when the tissue was frozen, samples of tomato leaves and beet leaves (without midribs) were ground as recommended by GILBERT. After extracting the juice which could be expressed by hand pressure, and centrifuging, the nitrate nitrogen was determined by the modified method. The ground residue remaining in the cloth was immediately frozen with solid CO_2, and allowed to stand overnight in the frozen state. Sixteen hours later this residue was thawed, pressed at once in the hydraulic press, centrifuged, and the nitrate nitrogen determined in the juice in the same manner. The averages of two closely agreeing determinations are given in each case:

CROP	LEAVES		DIFFERENCE
	GROUND NO_3-N	FROZEN NO_3-N	
	ppm.	ppm.	per cent.
Tomato	341	435	47.8
"	400	472	36.3
"	537	576	9.5
Beet (midribs removed)	310	411	37.7

It is evident that the discrepancies noted in table VIII may be attributed in part, at least, to the differences which may exist in the nitrate nitrogen content of the juices obtained by the two procedures. The juice obtained when the plant tissue is ground does not seem to be a true aliquot of the juice as it exists in the plant, and it is evident that the values obtained when the juice is secured by freezing will be more nearly correct. It is to be noted, however, that in the above experiments the differences between the two results are exaggerated, since the removal of a less concentrated aliquot tends to concentrate any fractions which may be removed subsequently. Hence the data shown indicate too high values for the frozen aliquot.

Summary

A considerable number of alterations have been made in the technic of estimating nitrate nitrogen in the juice of crop plants as previously recommended from this laboratory. It is recommended that the plant tissue be frozen and pressed after thawing rather than ground and squeezed through cloth by hand. The carbon black for decolorization has been omitted, and the quantities of reagents used for clearing the juice have been changed. A study of the recovery of nitrate nitrogen added to plant juices by the new method indicate rather wide variations, with the average of 25 determinations at 90.05 per cent.

The author wishes to acknowledge the extremely helpful criticisms of Mr. J. B. SMITH.

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LITERATURE CITED

1. EMERSON, PAUL. The colorimetric determination of soil nitrates in a colored water extract. Soil Sci. **12**: 413–417. 1921.
2. EMMERT, E. M. The determination of nitrate in green tomato and lettuce tissues. Plant Physiol. **4**: 519–528. 1929.
3. GILBERT, B. E. The adaptation of certain colorimetric methods to the estimation of nitrates, phosphates and potassium in plant solutions. Plant Physiol. **1**: 191–199. 1926.
4. GORTNER, R. A., and HARRIS, J. A. Studies on the physico-chemical properties of vegetable saps. 3. A comparison of the physico-chemical constants of the juices expressed from the wall with those from the included carpellary whorl in proliferous fruits of *Passiflora gracilis*. Biochem. Bull. **4**: 52–79. 1915.
5. HARPER, H. J. The accurate determination of nitrates in soils. Jour. Ind. Eng. Chem. **16**: 180–183. 1924.

6. HARVEY, R. B.   Hardening process in plants and developments from frost injury. Jour. Agr. Res. 15: 83–112. 1918.

7. HOLTZ, H. F., and LARSON, CARL.   A simple method for nitrate nitrogen determination in wheat plants. Plant Physiol. 4: 288–291. 1929.

8. KORSTIAN, C. F.   Density of cell sap in relation to environmental conditions in the Wasatch Mountains of Utah. Jour. Agr. Res. 28: 845–907. 1924.

9. LEWIS, F. J., and TUTTLE, G. M.   Osmotic properties of some plant cells at low temperatures. Ann. Bot. 34: 405–416. 1920.

10. LIPMAN, C. B., and SHARP, L. T.   Studies on the phenoldisulphonic acid method for determining nitrates in soils. Univ. California Pub. in Agr. Sci. 1: 21–37. 1912.

11. MEYER, B. S.   Seasonal variations in the physical and chemical properties of the pitch pine, with especial reference to cold resistance. Amer. Jour. Bot. 15: 449–472. 1928.

12. NEWTON, R., BROWN, W. R., and MARTIN, W. M.   The extraction of plant tissue fluids and their utility in physiological studies. Plant Physiol. 1: 57–67. 1926.

THE EFFECT OF FERTILIZER ON THE QUALITY AND KEEPING QUALITY OF WATERMELONS[1]

W. D. KIMBROUGH

Introduction

There is a belief among many people in the South that the quality of watermelons is affected by the fertilizer treatment given the soil on which the plants are grown. Many people believe that melons from plants fertilized with nitrate of soda are not sweet. Some believe that when melons, which have been fertilized with this source of nitrogen, are eaten, the consumer is likely to be made ill. The claim is made that melons from plants fertilized with nitrate of soda can be detected by taste. Buyers often discriminate against melons which have been fertilized with nitrate of soda. The reason given is that the melons are likely to have white heart, and be of poor quality.

Manure is generally considered an excellent fertilizer for melons and was used almost exclusively while it was cheap and easily obtainable. As manure becomes scarcer and more expensive, commercial fertilizer is being used more extensively in the melon fields. This makes it important that information concerning the influence of fertilizers on watermelons be obtained. For this reason, an experiment was started at the Alabama Experiment Station, in 1926, to determine the effect of fertilizer treatment on the quality and keeping quality of watermelons.

Review of literature

BEATTIE (1) states that some of the melon growers' associations of Texas prohibit the use of nitrate of soda as a top dressing, as they consider that it makes the melons soft and of poor carrying quality. JONES and ROSA (2) state that the claim is often made that potassium, applied in manure, favorably affects the flavor, quality, or sugar content of watermelons. They further say that the literature does not afford any evidence for such belief; but that potassium is a nutrient no doubt profitably applied in some cases. STUCKEY (3) reports that the results obtained for one year showed that the amount or type of white heart could not be attributed to the kind of fertilizer used. Unfavorable weather conditions during the fruiting period seemed to be the chief cause of white heart. Judged by taste, melons from plots receiving different fertilizer treatments gave no indication that the quality of melons was influenced by fertilizer treatment.

[1] Work on certain phases of this problem is being continued.

Procedure and methods

Watermelons were grown on well drained Norfolk sandy loam. The location of plots was changed three out of four years to avoid serious injury from disease. The first two years the same area was used and the plots were 1/40 acre with plants 6 feet apart each way. The third year plots were 1/10 acre and the fourth year 1/20 acre with plants 8 feet apart each way. Plots were separated by 3-foot alley ways. The third year the plots were on new ground, while the other years old land was used that had not been fertilized for a number of years. The variety "Wondermelon" was grown the first year and "Tom Watson" was grown the other three years. Melons were thinned to two to a vine.

Fertilizer treatments were run in duplicate, while check plots received no fertilizer. Fertilizer treatments are indicated by N, P, and K or multiples of these. The explanation of the symbols used is: N = 200 pounds of nitrate of soda, or equivalent per acre; P = 400 pounds of superphosphate per acre; K = 50 pounds of muriate of potash or equivalent per acre. Sources of fertilizer named were used unless otherwise stated. All fertilizer, except the nitrate of soda that was applied as top dressings, was applied broadcast before planting the first three years, and the fourth year it was applied along the rows in strips about four feet wide. Nitrate of soda was applied at times varying from entire application before planting to as many as four top dressings during the season. Nitrate of soda was not applied in amounts exceeding the rate of 200 pounds per acre or less than 100 pounds per acre at one application. Top dressings of nitrate of soda were applied to some plots after many melons were approximately half grown. Fertilizer applications were so varied in rate and sources, and in the time of application of nitrate of soda, that if fertilizer treatment affects the quality or keeping quality of melons this should have been true of melons produced in this experiment.

Quality was determined by taste and by determining the sugar and moisture content of the edible portion of melons. Judged by looks, only melons that were ripe and in good condition were sampled. In taking samples for analysis a portion of the melon extending from the rind to the center of the heart was freed of seed and ground in a food chopper. Samples for sugar determinations were preserved in alcohol in the usual way. Individual melons from a plot were sampled, rather than taking a sample from a group of melons. Only melons weighing 20 pounds or more were sampled for analysis.

Moisture content was determined by drying thoroughly mixed samples of material to constant weight in a vacuum oven at 80° C. and 26 inches of mercury. Reducing sugars were determined and expressed as glu-

cose, and total sugars as invert sugar. Inversion was brought about by acid hydrolysis at room temperature. Reducing sugars were determined by the Bertrand modification of the Munson and Walker method.[2]

Keeping quality was determined by storing melons at room temperature and at 35, 40, and 50° F. The storage temperature range was in general 2° F. each way from that given.

Experimental results

EFFECT OF FERTILIZER ON PLANT GROWTH

The plants showed a marked response in growth to the applications of fertilizer. Check plots produced plants that grew very slowly, but in some cases very good melons were eventually produced. The addition of both nitrogen and phosphorus was necessary for good growth, while the addition of potash along with these was necessary for best growth. Plants on plots receiving 2N made better growth than those on plots receiving 1N, but the addition of more than 2N made no apparent difference in plant growth. The third year of the experiment heavy rains apparently leached out nitrate of soda applied before planting to such an extent that plants got no benefit from it. Plants on manure plots made good growth but not as good as those on plots receiving 2N. Melons were a little earlier on manure and cottonseed meal plots than on plots receiving nitrate of soda as a source of nitrogen.

The response in plant growth to fertilizer treatment of the soil showed that the materials added were being utilized and indicated that if melons could be influenced by fertilizer treatment, this influence should have been obtained in melons produced on these plots.

EFFECT OF FERTILIZER ON QUALITY OF WATERMELONS

No laboratory was available in 1926, so quality of melons was determined by taste only. Three watermelon cuttings were held that were participated in by many members of the station staff and a few others. Melons from plots receiving different fertilizer treatments were cut and sampled. Those tasting the melons did not know the fertilizer treatment of the plots on which the melons were grown. The results obtained showed that there was apparently as much difference in melons from the same plot as there was between melons from plots receiving different fertilizer treatments. There was considerable difference of opinion as to which melons were best

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[2] Sugar content of the juice from the edible portion of several melons was determined with a Brix hydrometer and compared with the sugar content of the edible portion determined by the Bertrand modification of the Munson and Walker method. The average of determinations from nine melons showed that the Brix hydrometer method was about 1 per cent. higher than the other method.

but there was good agreement as to which melons were good, which were medium, and which were only fair in quality. Melons of good quality were produced from all treatments. The results of the watermelon cuttings indicated that the source of fertilizer did not materially influence the taste of the melons. After the tasting was over many ate large quantities of melons, including those that had been heavily fertilized with nitrate of soda, but no one became ill. During the period of four years, there was no report of melons from any plot being injurious to the consumer.

TABLE I

MOISTURE AND SUGAR CONTENT OF WATERMELONS FROM PLOTS RECEIVING DIFFERENT FERTILIZER TREATMENTS IN 1927

FERTILIZER TREATMENT	MOISTURE		SUGAR—FRESH WEIGHT			
			REDUCING		TOTAL AS INVERT	
	INDIVIDUAL MELONS	AVERAGE	INDIVIDUAL MELONS	AVERAGE	INDIVIDUAL MELONS	AVERAGE
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Check	90.26		3.95		8.95	
	90.54		4.90		7.98	
	90.71		4.63		7.73	
		90.50		4.49		8.05
NP	89.79		4.47		8.69	
	91.43		4.40		7.30	
		90.61		4.58		8.00
NPK	90.44		4.98		8.11	
	90.84		5.00		7.89	
		90.64		4.99		8.00
NPK (N from urea)	90.44		5.10		8.00	
	—		4.59		7.90	
				4.84		7.95
NPK (K from K₂SO₄)			5.18		8.35	
	90.27		4.57		8.21	
				4.87		8.28
2N2P2K	90.33		4.53		8.18	
	90.04		3.93		8.10	
	♠	90.18		4.23		8.14
3N2P2K	89.93		5.45		8.40	
	91.20		4.53		7.34	
		90.56		4.99		7.87
2NP2K			4.31		8.18	
	90.15		5.31		8.17	
				4.81		8.17
N2P2K	90.56		4.88		8.22	

The last three years of the work a laboratory was available and determinations were made to find the effect of fertilizer treatment on the moisture and sugar content of watermelons. At the time samples for analysis were taken the melons were tasted for quality by the writer and at least one assistant.[3] In general, the quality of melons judged by taste varied directly with the sugar content.

TABLE II
MOISTURE AND SUGAR CONTENT OF WATERMELONS FROM PLOTS RECEIVING DIFFERENT FERTILIZER TREATMENTS IN 1928

FERTILIZER TREATMENT	MOISTURE		SUGAR—FRESH WEIGHT			
			REDUCING		TOTAL AS INVERT	
	INDIVIDUAL MELONS	AVERAGE	INDIVIDUAL MELONS	AVERAGE	INDIVIDUAL MELONS	AVERAGE
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Manure (rate of 7.5 tons per acre)	89.68		3.68		8.47	
	90.10		3.77		8.15	
	90.56		3.59		7.80	
	90.74		3.77		7.60	
	91.39		4.05		7.12	
	91.63		4.45		6.99	
	92.12		4.35		6.63	
		90.89		3.95		7.52
NPK	89.78		5.19		8.27	
	90.20		3.90		7.91	
	90.49		4.09		7.90	
	90.51		4.42		7.90	
	90.55		4.36		7.82	
	91.78		4.79		6.77	
	92.44		4.59		6.17	
		90.82		4.48		7.54
4N2P2K	89.52		4.58		8.53	
	89.52		4.26		8.37	
	89.91		3.94		8.30	
	90.38		4.31		7.78	
	90.51		3.86		7.61	
	90.80		4.45		7.48	
	91.38		4.99		7.11	
		90.37		4.33		7.88
3N2P2K	89.71		4.36		8.47	
	89.92		4.58		8.29	
	90.77		4.45		7.57	
		90.13		4.46		8.11
2N2P2K	90.28		4.36		7.89	
	90.65		4.59		7.77	
	90.41		4.45		7.47	
	91.92		4.72		6.55	
		90.81		4.70		7.42

[3] After tasting a number of melons it was difficult to make fine distinctions in quality.

Results of analyses are given on individual melons as well as averages. This is done in order that the actual variation in the melons may be seen. In this way the relation between moisture content and sugar content is

TABLE III

MOISTURE AND SUGAR CONTENT OF WATERMELONS FROM PLOTS RECEIVING DIFFERENT FERTILIZER TREATMENT IN 1929

| FERTILIZER TREATMENT | MOISTURE | | SUGAR—FRESH WEIGHT | | | |
| | | | REDUCING | | TOTAL AS INVERT | |
	INDIVIDUAL MELONS	AVERAGE	INDIVIDUAL MELONS	AVERAGE	INDIVIDUAL MELONS	AVERAGE
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Check	89.35		4.05		8.31	
	90.19		3.85		7.96	
	90.02		3.82		7.78	
	90.48		4.34		7.72	
	90.60		3.81		7.58	
	91.13		4.38		7.07	
		90.33		4.04		7.74
Manure (at rate of 6 tons per acre)	90.03		3.53		7.91	
	89.94		3.50		7.87	
	90.44		3.86		7.53	
	90.73		3.62		7.32	
	90.97		3.95		7.17	
	91.52		4.43		6.65	
		90.60		3.81		7.41
2N2P4K	89.12		3.95		8.49	
	89.69		4.00		7.87	
	89.70		3.95		7.82	
	90.14		4.10		7.68	
	91.68		4.17		6.56	
		90.07		4.03		7.68
2N2P4K (K from K₂SO₄)	89.43		4.63		8.69	
	89.44		3.64		8.27	
	89.85		4.69		8.19	
	89.49		3.89		8.12	
	92.05		3.69		6.26	
		90.06		4.13		7.91
2N2P	89.96		4.53		8.11	
	90.08		3.73		7.92	
	90.49		4.46		7.62	
	90.62		3.21		7.50	
	90.41		4.39		7.48	
		90.31		4.06		7.73
2N2P4K (N from C. S. M.)	89.77		4.80		8.17	
	90.30		4.11		7.92	
	91.14		4.38		7.38	
	91.02		4.00		7.35	
	90.63		4.63		7.26	
		90.57		4.18		7.62

also clearly shown. The results secured in the different years are presented separately because of seasonal differences and differences in fertilizer treatments. Results are presented only from those plots that received fertilizer treatments most likely to affect the melons. The most important comparisons are between melons from the heavily nitrated plots and those from check plots, manure plots, and plots receiving cottonseed meal as a source of nitrogen. Results are given in tables I, II, and III.

The data in tables I, II, and III show that melons having high sugar content were obtained regardless of the fertilizer treatment. Melons from plots receiving heavy application of nitrate of soda had as high sugar content as melons from check, manure, and cottonseed meal plots. The rate or time of application of nitrate had no appreciable effect on sugar content. Potash fertilizers had no apparent influence on the sweetness of melons. There was approximately as much variation in moisture and sugar content in melons receiving the same fertilizer treatment as in melons from plots receiving different fertilizer treatment.

The consistency of the inverse relationship of sugar and moisture content is interesting. The sum obtained by adding the percentage of moisture and of sugar is almost constant.

EFFECT OF FERTILIZER TREATMENT ON THE KEEPING QUALITY OF WATERMELONS

The first two years of the experiment melons from plots receiving different fertilizer treatments were kept at room temperature. No apparent difference in keeping quality due to fertilizer treatment was found. Melons were kept as long as six weeks without rotting, but when cut the flavor was such that they were not eatable.

Sugar and moisture content of a few melons that had been stored at room temperature, were determined. The results obtained from melons that had been stored for 14 and 18 days respectively are shown in table IV.

If tables IV and I are compared it will be seen that there was a consistent decrease in sugar content and an increase in moisture content in the stored melons. The average moisture and sugar contents of similar melons sampled the days of harvest and after being held at room temperature for 14 and 18 days respectively are shown in table V.

Melons cut after being kept from one to three weeks at room temperature seemed redder in color and had thinner rinds than melons cut immediately after harvest. This might indicate that melons continue to ripen after harvest but the quality of the melons cut the day of harvest was better than that of melons kept at room temperature for several days after harvest. The sugar content has been shown to be decreased when melons are kept at

TABLE IV

MOISTURE AND SUGAR CONTENT OF MELONS KEPT AT ROOM TEMPERATURE

FERTILIZER TREATMENT	SAMPLED 14 DAYS AFTER HARVEST			SAMPLED 18 DAYS AFTER HARVEST		
	MOISTURE	SUGAR		MOISTURE	SUGAR	
		REDUCING	TOTAL AS INVERT		REDUCING	TOTAL AS INVERT
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Check	90.79	3.28	7.48	91.09	3.37	7.31
NPK	90.85	3.36	7.15	92.13	3.21	6.39
N2P2K	91.22	3.41	7.20	91.53	3.41	6.94
2N2P2K ·..........	91.69	2.84	6.65	91.93	3.36	6.65
3NPK	91.81	3.80	6.52	92.00	2.71	6.60
3N3P3K	91.68	3.80	6.82			
2NP2K	91.09	2.89	7.19			
3N2P2K	91.35	3.80	6.82			

room temperature, so it is doubtful if melons ever get sweeter after they are harvested. The increase in moisture content shown in table V is not large, but it evidently occurs. The loss of moisture due to transpiration is not enough to balance the loss in sugar content plus the moisture formed in the respiratory process.

TABLE V

COMPARISON OF AVERAGE MOISTURE AND SUGAR CONTENT IN MELONS SAMPLED THE DAY OF HARVEST AND MELONS KEPT AT ROOM TEMPERATURE

TIME OF SAMPLING	MOISTURE	SUGAR		DECREASE IN SUGAR CONTENT
		REDUCING	TOTAL AS INVERT	
	per cent.	per cent.	per cent.	per cent.
Day of harvest	90.47	5.09	8.03	
14 days after harvest	91.31	3.32	6.95	13.45
Day of harvest	90.69	4.47	8.20	
18 days after harvest	91.73	3.21	6.77	17.44

In 1928 and 1929 melons were stored at temperatures of 35, 40, and 50° F. There was no apparent difference in keeping quality due to fertilizer treatment.[4] The lower the temperature the better the melons kept. Melons kept for two months at 35° F., however, were in bad condition internally even though they seemed in fair condition externally. It seems that in order to store melons for long periods a temperature below 35° F. is necessary.

TABLE VI

MOISTURE AND SUGAR CONTENT OF MELONS STORED AT 40° F. FOR ONE MONTH

FERTILIZER TREATMENT	MOISTURE	SUGAR—FRESH WEIGHT	
		REDUCING	TOTAL AS INVERT
	per cent.	per cent.	per cent.
Manure	90.09	3.18	8.13
	90.60	2.59	7.69
	91.19	2.87	7.23
2N2P2K	90.22	2.80	7.89
	90.51	2.97	7.78
	90.81	3.19	7.48

TABLE VII

MOISTURE AND SUGAR CONTENT OF MELONS STORED AT 35° F. FOR ONE MONTH

FERTILIZER TREATMENT	MOISTURE	SUGAR—FRESH WEIGHT	
		REDUCING	TOTAL AS INVERT
	per cent.	per cent.	per cent.
Manure	90.61	2.42	7.58
2N2P2K	90.06	3.31	8.13
	91.39	4.37	6.44
3N2P2K	89.80	3.25	8.09
	91.41	4.25	6.95
PNK	89.93	2.92	8.08
	90.50	2.33	7.69
Check	89.27	3.33	8.62
	90.58	3.90	7.64
	90.68	3.11	7.53

[4] In 1928, symbols indicating the fertilizer treatment of the plot from which the melon came were scratched on the melons. This proved a means of entrance for decay organisms.

Moisture and sugar contents of some melons stored for a month at 35° F. and 40° F. respectively were determined. Results obtained are shown in tables VI and VII. These tables show that there is probably very little difference in the moisture and sugar content of the stored melons and freshly harvested melons, except that the reducing sugars are apparently decreased slightly. In storage, part of the reducing sugars seem to be changed to non-reducing sugars. When cut after removal from storage, the melons were lighter red than melons kept at room temperature.

EFFECT OF FERTILIZER ON WHITE HEART IN MELONS

Due to the limited number of melons during the first two years that the experiment was conducted there was no special cutting of melons to examine for white heart. Most of the melons produced were cut for various reasons, however, and no white hearted melons were noted.

TABLE VIII

MELONS EXAMINED FOR WHITE HEART AND PERCENTAGE FOUND IN 1928

FERTILIZER TREATMENT	NUMBER OF MELONS EXAMINED	NUMBER WITH WHITE HEART	WHITE HEARTED MELONS
			per cent.
Check	35	3	8.6
2P2K	10	0	0.0
N2P2K	57	3	4.9
2N2P2K	99	13	13.1
3N2P2K	83	8	9.7
4N2P2K	72	5	7.0
Manure	57	7	12.3

TABLE IX

MELONS EXAMINED FOR WHITE HEART AND PERCENTAGE FOUND IN 1929

FERTILIZER TREATMENT	NUMBER OF MELONS EXAMINED	NUMBER WITH WHITE HEART	WHITE HEARTED MELONS
			per cent.
Check	30	6	20.0
NP2K	33	5	15.1
N2P2K	31	2	6.5
2N2P2K	25	1	4.0
2N2P4K	54	9	16.7
2N2P4K (K from K_2SO_4)	31	3	9.7
2N2P4K (N from cottonseed meal	21	2	9.5
Manure	27	5	14.8

The last two years, 1928 and 1929, a number of melons from plots receiving different fertilizer treatments was examined for white heart. Melons having a distinct white streak in the middle and otherwise ripe, were considered to have white heart. The results obtained are shown in tables VIII and IX.

There does not seem to be any correlation between fertilizer treatment and white heart in melons. There was no higher percentage of melons with white heart from plots receiving heavy applications of nitrate of soda than from plots receiving manure or from the no fertilizer plots. The fertilizer treatment seems to have little, if any, effect on the production of white heart in melons. Most of the white hearted melons were found late in the season when unfavorable climatic conditions or disease had injured the vines.

Discussion

The results of four years work on the effect of fertilizer treatment on the quality and keeping quality of watermelons have been presented. The seasons have been variable enough to cover quite a range of climatic conditions. Three different plot areas were used and while all were of a light sandy nature, and well drained, they were somewhat variable. The response in plant growth to the fertilizer applied was usually striking, and showed that the plant was making use of the fertilizer. Especial emphasis was placed on the effect of nitrate of soda on the water-melons and this was applied at such varying rates and times that the effect, if any, should have been detected. Quality of melons was determined by taste and by analysis for moisture and sugar contents of the edible portion. Melons from heavily nitrated plots were compared especially with melons from check plots, manure plots, and plots receiving cottonseed meal as a source of nitrogen. The moisture and sugar contents of 175 individual melons were determined over a period of three years. Melons were stored at room temperature and in cold storage rooms at temperatures of 35, 40, and 50° F. to determine keeping qualities.

The data presented show no apparent difference in the quality of melons due to fertilizer treatment. The sugar content of melons varied as much between melons of the same treatment as those of different treatments. Melons from plots receiving heavy applications of nitrate of soda and applications late in the season had as high sugar content as melons from manure, cottonseed meal, or check plots. Potash fertilizers did not increase the sugar content. Melons from plots receiving nitrate of soda did not cause sickness when eaten. There was no indication that white heart in watermelons was correlated with fertilizer treatment. White heart was not usually found unless dry weather or disease had injured the vines.

There was no indication that fertilizer treatment of plants affected the keeping of the melons.

In general, quality, determined by taste, agreed very well with the sugar content, the better quality melons having the higher sugar content. Sugar and moisture content varied inversely. The sugar and moisture content relationship was such, in fact, that moisture content will give a fair estimate of sugar content.

Melons kept at room temperature for periods of one to nearly three weeks showed consistently a slight increase in moisture content and a decrease in sugar content. The decrease in sugar content is largely in the reducing sugar. The rapid decrease in sugar content in melons at room temperature is a partial explanation of the difference between shipped and home grown melons which are eaten soon after harvest. The rapid decrease in sugar at room temperature is also further evidence that the supposed ripening of melons which are kept for several days in order to improve them does not occur. The Tom Watson melon is not generally praised because of its sweetness, but many melons of this variety were found to have a high sugar content.

Summary

1. On sandy soil no apparent influence of fertilizer treatment on quality of watermelons was found.

(a) Nitrate of soda did not prevent melons from having a high sugar content and being sweet.

(b) Potash did not increase the sugar content.

2. The edible portion of the watermelon is about 98 per cent. sugar and water. The moisture and sugar content vary inversely with each other.

3. After harvest the sugar content of melons kept at room temperature decreases rather rapidly.

4. Moisture content may increase slightly in melons kept at room temperature.

5. No effect of fertilizer treatment on keeping quality of watermelons was found.

6. Temperature as low as 35° F. was not effective for keeping melons in good condition for much over a month.

7. No correlation between fertilizer treatment and white heart in melons was found.

8. White heart seems to be caused by unfavorable climatic conditions or disease injury to the vines.

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LITERATURE CITED

1. BEATTIE, W. R. Watermelons. Farmer's Bull. 1394. 1927.
2. JONES, H. A., and ROSA, J. T. Truck crop plants. McGraw-Hill. New York. 1928.
3. STUCKEY, H. P. Watermelons. Georgia Agr. Exp. Sta. Bull. 143. 1924.

THE ESSENTIAL NATURE OF BORON TO THE GROWTH AND FRUITING OF THE TOMATO

EARL S. JOHNSTON AND PAUL L. FISHER

(WITH THREE FIGURES)

A question that frequently arises in connection with elements regarded necessary for plant growth is whether or not a particular element is an essential plant food or merely a stimulating substance. Is its rôle that of a catalyzer, or is it actually incorporated into the tissues as an integral part of the plant? These questions are more applicable to such elements as manganese, copper, zinc and boron than to nitrogen, phosphorus and potassium, which have been recognized for many years as essential nutrient units. The elements of both groups have at times been considered toxic, at others as stimulating. Here again confusion exists, for the question of toxicity seems to depend upon circumstances. Thus BRENCHLEY (1) points out that ''Typical nutrient salts are toxic when they are applied singly to the plant in certain concentrations, the toxic power decreasing and the nutritive function coming into play more fully on the addition of other nutrient salts.'' Some elements which are toxic in high concentrations contribute to increased plant growth when their solutions are properly diluted. It is apparent then that no sharp distinction can be drawn between toxic elements, and nutrient elements, or stimulating elements if such exist. The distinction is a question rather of quantity than of kind, which should be considered in connection with other factors such as the presence of other elements. BRENCHLEY (1) has divided the class of elements that are toxic in high concentrations into two groups: ''(1) Those that apparently become indifferent in high dilutions and never produce any increase in plant growth; (2) Those that are either essential for growth, or at least cause increased development, when applied in sufficiently small quantities. The former group may be legitimately regarded as toxins; the latter presents more difficulty and even now their function is not settled. It is not clear whether they stimulate the protoplasm or in some way hasten the metabolic processes in the plant, whether they help roots in their absorbent work, or whether they are simple nutrients needed only in infinitesimal quantities.''

Boron without a doubt belongs in the second group. Its rôle in the plant is not known although several investigators have shown that its presence is essential for the normal growth of many plants. WARINGTON (7) states, ''The fact that boron can be detected in the stem, leaves, and pods of the broad bean implies that the element becomes distributed throughout

the plant after absorption; and, further, the need for the supply of boron
to be maintained during the life of the plant indicates that the initial re-
serve of the element in the seed is insufficient for the needs of the plant,
and that it is in some way fixed and not in a state of circulation." If this
be true it would then appear that boron does not act in the capacity of a
catalizer, but that its rôle is similar, though to a much less extent, to those
of other elements classified as essential.

"The old 'nutrients'," as BRENCHLEY (1) states, "had certain definite
characters in common, in that they were essential to plant growth, the
growth being in a great degree proportional to the supply, a relatively large
amount of the nutrients being not only tolerated but necessary." Evidence
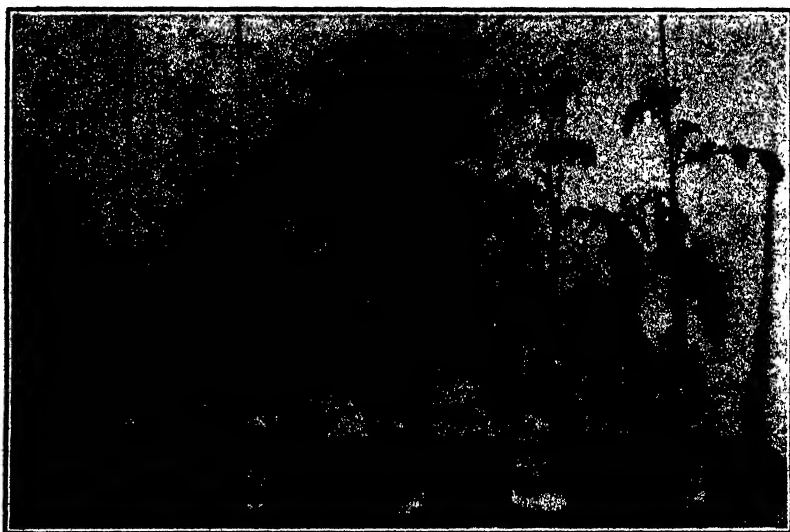
FIG. 1. Tomato plants grown in similar nutrient solutions to which different
amounts of boron as boric acid had been added. (Left to right: 0.0; 0.22; 0.11; 0.55;
2.75 ppm. respectively.)

has been presented by JOHNSTON and DORE (5) which shows the possibility
of a quantitative relationship existing between the amount of growth and
the amount of boron present in the nutrient media. Further evidence was
found in the growth of tomato plants reported by NEWELL (6) and illus-
trated in figure 1. An increase in concentration of boron from 0.55 ppm. to
2.75 ppm. produced a slight decrease in height in most cases. For concen-
trations below 0.55 ppm., with the possible exception of 0.11 ppm., a sharp
decrease in height was evident. In general, these same relations also held
for the green and dry weights.

Evidence is presented in the present paper which indicates the necessity
for an available and constant source of boron supply in order that good

growth may be maintained throughout the life cycle of the tomato plant. Prior to these experiments it was thought that a sufficient quantity of boron, which is needed in almost infinitesimal amounts, might possibly be absorbed during the early stages of growth to amply supply the needs of the plant through the later or fruiting stage.   The work of GERICKE (3, 4) and of BRENCHLEY (2) indicates that this is true for some of the recognized nutri-

FIG. 2.   Tomato plants grown in similar nutrient solutions to blossoming, at which time the plant on the right was changed to a boron deficient solution.

ent elements in the case of wheat and barley.   An experiment was carried out to ascertain the necessity of a continuous boron supply for the tomato plant.

Forty Marglobe tomato seedlings were set out in one-gallon culture jars, one plant per each jar, and supplied with a nutrient solution of the following volume-molecular concentration:

$$Ca(NO_3)_2 \quad \text{...} \quad 0.005$$
$$MgSO_4 \quad \text{...} \quad 0.002$$
$$KH_2PO_4 \quad \text{...} \quad 0.002$$

To this general nutrient solution manganese as $MnSO_4$ was added at the rate of 1 ppm. and boron as H_3BO_3 at the rate of 0.5 ppm. While the plants were young ferric tartrate (0.5 per cent. solution) was added daily at the
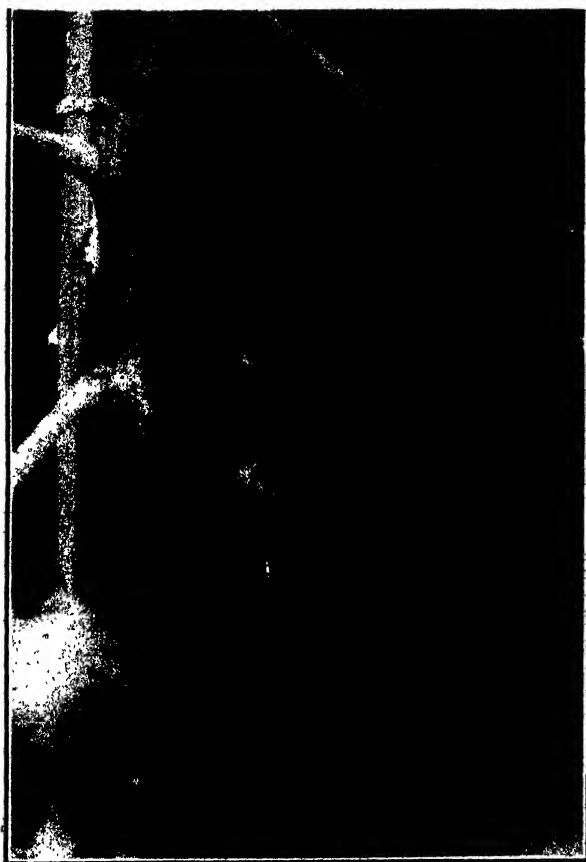
FIG. 3. Dark dead areas appear on the fruit of tomato plants grown in boron deficient solutions during the fruiting phase of growth.

rate of a cubic centimeter per liter of nutrient solution. After the roots were well developed the iron was added less frequently. The experiment was started on October 9, 1929. All the plants used were healthy and of uniform size and continued to remain so under similar treatments.

In the above solution and under similar greenhouse conditions the plants were grown until December 6. At this date three-fourths of the plants were

in blossom.   These cultures were then divided into two groups and the solutions renewed.   Group *A* plants were placed in a boron deficient solution while the solution of group *B* plants contained 0.5 ppm. boron the same as the original solution of all the plants.   The average stem heights (usually taken at weekly intervals) for the two groups are given in table I.   This

TABLE I

DATA SHOWING TOTAL STEM HEIGHT AND GREEN AND DRY WEIGHTS OF TOMATO PLANTS,
EXPRESSED AS THE AVERAGE PER PLANT PER EACH GROUP

DATE OF OBSERVATION	GROUP A*	GROUP B
1929	*cm.*	*cm.*
October 17	2.5	2.4
" 24	5.0	4.6
" 31	7.4	7.3
November 7	13.7	14.0
" 14	23.5	24.3
" 22	39.5	42.3
" 29	49.7	52.9
December 7	56.2	59.1
" 16	63.5	66.5
" 22	81.1	87.1
" 29	82.2	90.8
1930		
January 3	82.7	99.8
" 17	82.2	101.3
	gm.	*gm.*
Green weight:		
Tops	143.3	146.3
Roots	52.5	59.2
Total	195.8	205.5
	gm.	*gm.*
Dry weight:		
Tops	16.8	19.5
Roots	4.3	5.4
Total	21.1	24.9

* On December 6, plants in group A were changed to a boron deficient solution while those of group B were changed to a similar solution to which boron was added (0.5 ppm.).

table also shows the average green and dry weights of the plants at the end of the experiment, January 17, 1930.   It is to be noted that approximately two weeks after group *A* plants were changed to a boron deficient solution their stem-height growth began to fall off.   Furthermore, the tops turned yellow and died in a manner similar to plants showing boron deficiency

symptoms at an earlier growth stage. The characteristic stem brittleness also made its appearance. The general appearance of representative plants of the two groups is shown in figure 2.

One of the interesting features of this experiment was the effect of a boron deficient solution on fruit setting and the characteristic appearance of such fruit that did develop. Approximately four times as many fruits set on the plants of group B as on those of group A. Fruits of the latter group were covered with darkened or dead areas which were apparently due to the breaking down of the cells making up this tissue. These dead areas were somewhat similar to those occurring on tomatoes injured by blossom end rot, with one exception however; the dead tissue did not necessarily begin at the blossom end of the fruit, but was scattered over the entire surface in a haphazard manner. While there was a certain similarity to blossom end rot yet the boron deficient symptom was quite different and distinct. The general appearance of representative fruits from a plant of group A is shown in figure 3.

It thus appears that for the tomato plant a constant source of boron is necessary for its normal growth, the setting of its fruit, and the development of its fruit. Boron is apparently fixed in plant tissues and cannot be used over and over again. It is equally important in the vegetative and the fruiting phases of growth. Boron appears to function as a simple nutrient element needed in extremely minute quantities.

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LITERATURE CITED

1. BRENCHLEY, WINIFRED E. Inorganic plant poisons and stimulants. 2nd ed. Cambridge University Press. 1927.
2. ——————. The phosphate requirement of barley at different periods of growth. Ann. Bot. **43**: 89–110. 1929.
3. GERICKE, W. F. The beneficial effect to wheat growth due to depletion of available phosphorus in the culture media. Science **60**: 297–298. 1924.
4. ——————. Salt requirements of wheat at different growth phases. Bot. Gaz. **80**: 410–425. 1925.
5. JOHNSTON, EARL S., and DORE, W. H. The influence of boron on the chemical composition and growth of the tomato plant. Plant Physiol. **4**: 32–62. 1929.
6. NEWELL, H. J. Range of boron concentrations in nutrient solutions for plants. (Unpublished.)
7. WARINGTON, K. The changes induced in the anatomical structure of *Vicia faba* by the absence of boron from the nutrient solution. Ann. Bot. **40**: 27–42. 1926.

ACCUMULATED IRON IN THE NODES OF CORN PLANTS[1]

J. D. SAYRE

(WITH ONE PLATE)

HOFFER and his colleagues (1, 2, 3) reported on the accumulation of iron in corn plants and suggested that this had a relation to the problem of root rots. HOFFER (4, 5) also suggested a relation between iron accumulation and potash deficiency, and that simple colorimetric tests for accumulated iron could be used to indicate potash needs. WELTON, MORRIS and GERDEL (8) did not find the relation between accumulated iron and potash deficiency close enough to permit using such tests to indicate need for potash fertilizer. SALTER and AMES (7) found a considerable variation in the percentage of total iron in the nodal tissues among individual corn plants. They found little or no relation between such quantitative determinations of total iron and the results of the colorimetric tests used by HOFFER. HOFFER and CARR (1) state that the accumulated iron occurs in the phloem of the vascular bundles.

This report is an attempt to find out more fully the form or kind of iron and its place of occurrence in the corn plant. No attempt is made to correlate iron accumulation with any mineral nutrient deficiencies.

Material and methods

The corn plants used in these investigations were of such varieties as happened to be growing under conditions where iron accumulation was high. Some plants from the vicinity of Wooster were used but usually had too little iron accumulation to be satisfactory. Corn from southwestern Ohio and from the muck lands at Lodi, Ohio, constituted the best material obtainable. Plants which were "fired" usually had the largest iron accumulations, but occasional plants without this symptom would contain a considerable amount. Discoloration of the nodal tissues served as a good preliminary indicator of iron accumulation, but was not infallible, as not all such discoloration is due to iron.

In addition to gross tests on the split stalks, the iron accumulations were studied microscopically, both in cut sections of the fresh stalks and in dried, pulverized nodal tissues. The sections were cut free hand with an iron-free knife. The dried tissue examined was first pulverized and passed over a

300 mesh sieve. The fine material passing this sieve contained the iron accumulations freed from much of the surrounding tissues.

The potassium ferrocyanide, $K_4Fe(CN)_6$, and potassium ferricyanide, $K_3Fe(CN)_6$, tests given by KLEIN (6) were used. These tests are specific for iron, the one $(K_4Fe(CN)_6)$ testing for ferric, and the other $(K_3Fe(CN)_6)$ for ferrous iron. The blue products formed do not diffuse readily through the tissue and thus show very well the localization of the iron. The tests are more or less permanent if the mounts are properly prepared. HOFFER and CARR (2) used the potassium thiocyanate (KCNS) test for iron in their work. This test is very sensitive and is specific for ferric iron. The ferric thiocyanate (FeCNS) formed in the test is soluble however, and soon diffuses throughout the tissue so that localization can not be determined. Furthermore it shows the presence of ferric iron only. For these reasons it was used only occasionally to check the results of the other methods.

None of these tests show the presence of masked or organic iron, but are for the free or inorganic forms, the only kind here considered.

Observations on accumulated iron

Iron occurs in different amounts in different plants. Very few plants were found which did not show a trace of iron at some of the nodes. There was a variation in the amount of iron at the different nodes of a single plant, the amount usually being largest at the ear node. The accumulated iron was found only at the nodal plate, where the veins anastomose and extend into the leaf sheath.

Under the microscope it was found that the iron occurs in the cells of the bundle sheath and in the first layers of pith cells around the bundle. In no case was accumulated iron found in the conducting elements of the bundles. This fact is best shown by watching the section under the microscope as the test is applied. In plants which had very little accumulated iron at the nodes, microscopical examination of a thin section of the tissue before treatment with potassium ferrocyanide revealed minute granules in those cells where iron accumulates. These granules were shown to be iron or to contain iron, by their reaction as they came into contact with the test solutions. Where the iron occurs in larger amounts, the cells are found to contain either large irregular masses, which are red to reddish brown in color, or crystals which are red in color, or both. These masses and crystals gave the reaction for iron.

After standing for some time in the reagents, the masses and crystals disappear, but the blue color indicative of iron remains, diffusing throughout the containing cells. The blue substance which is formed in the reaction, however, does not diffuse through the cell wall, but eventually may be

adsorbed by the cell wall, the cell contents becoming colorless.   Plate III, figures 1 and 2 show photo-micrographs of cross sections of a node of a corn plant having a large accumulation of iron.   The sections are of the fresh corn stem mounted in water only, not stained for iron.   The dark spots surrounding the bundles are the crystals and masses.

The crystals and masses test equally well for ferric or for ferrous iron. The masses are often as large as 30 μ and the crystals may be as large as 14–16 μ.   The cells containing them are 60–80 μ in diameter.

Optical properties of the crystals and masses

The crystals and masses are isotropic; they are dark under polarized light in all positions, and remain so as the stage of the microscope is rotated. They therefore belong to the cubic crystal system.   The refractive index was determined from material separated from dried, pulverized nodal tissue as already described.   This material contained relatively few of the masses and crystals but enough to permit determining their refractive index by means of immersion oils.   The refractive index of the crystals was 1.59 and that of the masses somewhat lower, 1.57–1.58.   The crystals may be square, rectangular, or hexagonal in outline and cubic or octahedral in form (plate III, figures 3, 4, 5, 6).

Behavior of the crystals and masses with solvents

No solvent has been found which will entirely remove the iron from the tissue.   The crystals disappeared in dilute acids and alkalies, in hot alcohol, in hot water, even in alcohol and glycerine in the cold after several days. The tissue which had been treated with these solvents still gave a very good test for iron, but only in those cells in which iron ordinarily occurs.   Only a trace of iron could be removed by extraction with hot alcohol in a Soxhlet extractor for 20 hours.   The explanation of this behavior seems to be that the substances are largely dispersed rather than dissolved.   Concentrated acids and alkalies destroyed the crystals and also the whole tissue.   The crystals and masses remained unaltered in tissue dried at ordinary temperatures.

Conclusion

A careful search of the literature failed to reveal a record of any compound of iron having the properties of these crystals and masses as detailed above.   The problem was taken to Professor W. G. McCaughey, mineralogist of the Ohio State University, for advice.   It was concluded that it is not a simple compound of iron, but probably a mixture or solid solution containing a compound of iron.   The crystal itself is probably some other substance, the host substance, which contains some form of iron mixed with it in solid solution.   Iron often occurs in this way, especially in organic

matter, and a trace of iron may color the host substance deeply, giving the appearance of a heavy deposit, or a pure compound of iron. Thus, no real measure of the quantity of iron present in such ways can be obtained from its color. The oxide and hydroxides of iron are the forms which usually occur in this way.

The properties of the crystals are similar to those of some of the protein crystaloids. Much more work would be needed however to permit any conclusions as to the nature of the host substance.

Summary

1. The iron studied in this work occurred only at the nodal plates of corn stems, and only in the bundle sheath and outer layers of pith cells around the bundle.

2. It accumulates as masses and crystals containing iron, which have definite optical and other properties.

3. The crystals and masses are isotropic. The crystals belong to the cubic crystal system, and have a refractive index of n = 1.59.

4. The iron is present probably not as a simple compound, but as an oxide or hydroxide of iron in solid solution in some host substance. The properties of the crystals suggest that the host substance may be protein in nature.

LITERATURE CITED

1. HOFFER, G. N., and CARR, R. H. Iron accumulation and mobility in diseased cornstalks (abstract). Phytopath. 10: 56. 1920.

2. —————, and —————. Accumulation of aluminum and iron compounds in corn plants in its probable relation to root rots. Jour. Agr. Res. 23: 801–823. 1923.

3. —————, and TROST, J. F. The accumulation of iron and aluminum compounds in corn plants and its probable relation to root rot. Jour. Amer. Soc. Agron. 15: 323–331. 1923.

4. —————. A simple test for detecting the nutrient needs of corn plants. Jour. Amer. Soc. Agron. 18: 29–31. 1926.

5. —————. Testing corn stalks chemically to aid in determining their plant food needs. Ind. Agr. Exp. Sta. Bull. 298. 1926.

6. KLEIN, GUSTAV. Praktikum der Histochemie. Julius Springer. Berlin. 1929.

7. SALTER, R. M., and AMES, J. W. Plant composition as a guide to the availability of soil nutrients. Jour. Amer. Soc. Agron. 20: 808–836. 1928.

8. WELTON, F. A., MORRIS, V. H., and GERDEL, R. W. Corn stalks vs. field plots as a guide to the fertility requirements of the corn crop. Ohio Agr. Exp. Sta. Bull. 397. 1926.

FIG. 1.   Cross section of node of corn showing iron accumulated around the vascular bundles (× 60).

FIG. 2.   Cross section of a vascular bundle at the node of corn showing iron accumulated around the bundle (× 110).

FIG. 3, 4, 5.   Crystals and masses containing iron at the node of the corn stem (× 260).

FIG. 6.   Crystal containing iron at the node of the corn stem (× 600).

All sections unstained, and mounted in water.

Fig. 1   Fig. 2

Fig. 3   Fig. 4   Fig. 5   Fig. 6
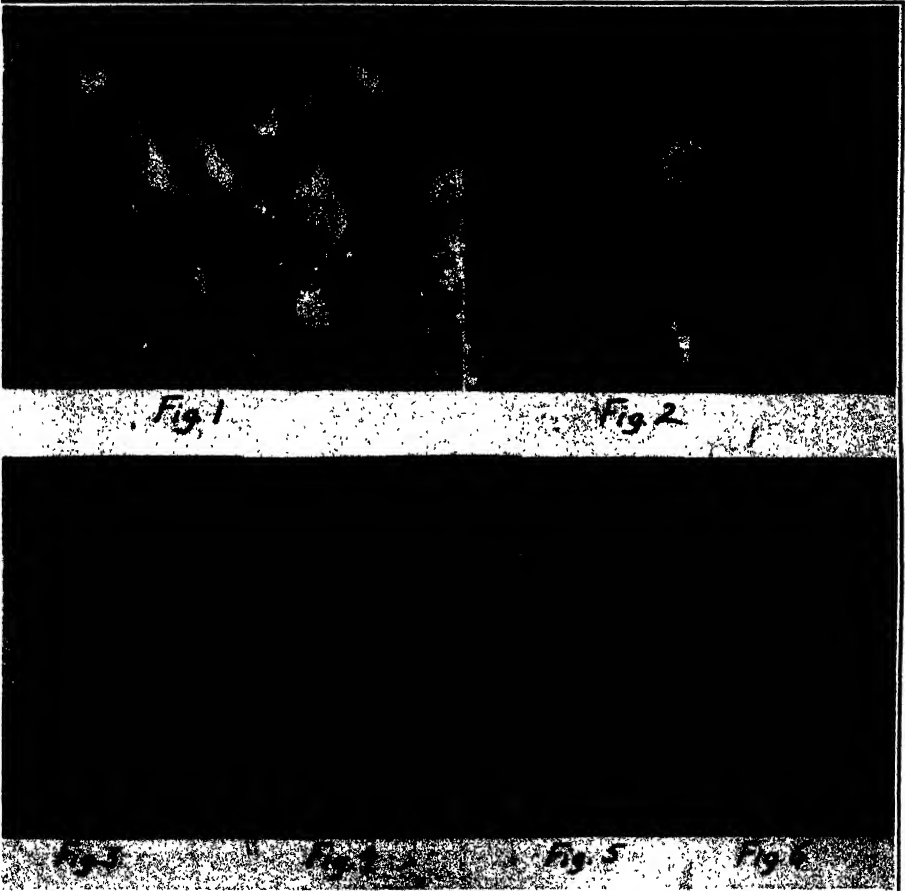
SAYRE—IRON ACCUMULATION

THE CHEMICAL CHANGES IN PEAS AFTER PICKING

ZOLTAN I. KERTESZ

(WITH TWO FIGURES)

It is a matter of common knowledge that unfavorable changes occur in the flavor of peas after they have been picked from the vine. "Only one hour from the field to the can" has become the watchword of the canners in their efforts to avoid these changes. It is the object of these studies to follow the changes by the determination of certain constituents of the peas.

It has been established that many parts of plants do not cease respiring upon being severed from the rest of the plant. Even though the respiration may be stopped by drying, if the moisture of the severed part is restored it will begin again. The investigations of PALLADIN showed that respiration continues even when the cells have been destroyed or killed in certain ways. This is called postmortal respiration (8) and an extensive literature dealing with it has already come into existence (3).

The chemical composition of peas at different stages of ripening was studied by BOSWELL (4). In his experiments it is shown that the total sugar content decreases during ripening. The starch content increases as does also the proportion of protein nitrogen. These findings are corroborated by the work of LÜNING and BEYER (11) who also demonstrated the increase in the proportion of starch and of protein.

There is, however, little to be found in the literature in regard to changes that may take place in peas after picking. Nevertheless, the question is of great importance, for in 1928 no less than 195,000 tons of peas were produced for canning purposes, and another considerable amount was marketed without canning. 78 per cent. of the latter were sold in New York and Philadelphia (13). Most of these peas must be shipped, involving a lapse of one to several days time before they can be placed in the hands of the consumer. These market peas, it should be mentioned, are left in the pods, and the changes in composition which they undergo are probably neither so rapid nor so extensive as those in shelled peas.

Work has been done on this problem with other plants, for example by ROSA (12) and IVANOFF and collaborators (9) on melons, on apples by BLACKMAN and PARIJA (2), etc., but no similar studies have been found for peas. They were not even included among the materials examined by BENOY (1). She studied the respiration of several vegetables (asparagus, lettuce, green beans, okra, green onions) harvested in edible condition, by determining the rate of carbon dioxide evolution in the first few hours after gathering. In plants where this rate is high to begin with it falls

rapidly, as in asparagus. Where the initial rate is moderate or small compared with the total observed range it falls slowly, and where the initial rate is very slight it remains almost constant for the first 25 to 30 hours. In her paper BENOY has calculated the amount of glucose corresponding to the amount of carbon dioxide produced by certain plants in the first 2 to 24 hours. This shows the amount of glucose the plant would have used up in respiration if this process had been carried on entirely at the expense of glucose present in the plant. Some of these values which will be referred to later, are as follows: asparagus, 13.68; green beans, 6.32; onions, 4.57; tomato, 2.67 gm. per 100 gm. of dry matter. LASAUSSE, GUERITHAULT and PELLERIN (10) determined the cellulose, total nitrogen and starch content of fresh and dried peas, but no conclusions can be drawn from this work regarding the changes in freshly picked peas.

The flavor of peas, both in pods and shelled, changed decidedly in the course of a few hours after picking. GOWEN (6, 7) writes that after three to four hours the flavor of peas is not as agreeable as of those freshly picked and that after eight hours they have become tougher. The observed changes in the chemical composition of peas are in all probability due to the activity of enzymes. From BENOY's experiments it may be deduced that green peas should be included among the products that show a comparatively high respiratory activity. This notion is supported by the observation of GOWEN that the temperature of peas left for 6–8 hours in a box after picking rises some 8 to 10° C.

The following experiments, which are to be regarded as preliminary, are intended to show the character of these changes. Of course, chemical analysis can not be expected to give a complete picture of the respiratory changes, but the object of these experiments was not to determine the amount of carbon dioxide produced, but to follow the changes taking place in the composition of the peas after picking. Nevertheless, the data obtained permit the drawing of certain conclusions regarding respiration.

Methods and materials

Samples of unsifted peas (variety "Rogers C") were picked at nine o'clock in the morning, taken to the laboratory, shelled by hand and weighed. The work was so arranged that none of the samples were allowed to remain in the pods more than one hour, and work was begun on them within ten to fifteen minutes after shelling. They were distributed into samples of about 30 gm. each. Two treatments were used: (1) One group of samples was placed in open dishes and left on the laboratory shelves for various periods of time. (2) Another group was macerated in a mortar, placed in an Erlenmeyer flask with 100 ml. of water containing 3 per cent. toluene, and allowed to stand for various periods of time.

After the lapse of fixed intervals of time the samples were prepared for analysis by boiling with 200 ml. of 95 per cent. alcohol for three minutes, allowed to cool, and stored in the refrigerator until the analysis was begun.

Upon removal from the ice-box the samples were ground, and the whole placed in a Soxhlet extraction apparatus and extracted with 500 ml. of 80 per cent. alcohol for several hours. The extraction was terminated as soon as a portion taken from the top of the thimble failed to give the MOLISCH (alpha-naphthol) reaction for sugar. The residue from the extraction was dried and weighed.

A 1.5 gram portion was taken for the determination of starch, and two 0.5 gram portions for the determination of protein nitrogen.[1] Reducing sugars, sucrose, and non-protein nitrogen were determined in the extract, the sugars by Bertrand's method. The procedure outlined by BOSWELL (4) was followed in general. His work indicated the necessity of using boiling alcohol to prevent the hydrolysis of sucrose.

Chemical changes in whole peas

The data secured on whole peas are contained in table I and figure 1. The solid lines show the changes in the peas on the vines and the dotted and dashed lines those in the peas during storage under the conditions described.

The dry matter content of the peas on the vines increases throughout the period studied.

The story of the carbohydrates as told by the chart is probably the most important. We can summarize the changes on the vine by saying that the sucrose increases rapidly for the first week after the blossoming period, remains on a level for a few days, and then decreases rather rapidly. Reducing sugars remain at a low level throughout, and starch increases gradually. The alcohol-insoluble residue which is largely carbohydrate, increases at first slowly, then very rapidly. After the peas are removed from the vines a different series of changes takes place. There is an immediate and striking reduction in sucrose in every case. On the other hand the reducing sugars and starch remain almost unchanged. The residue, however, undergoes as striking an increase as the sucrose does a decrease. The chart indicates that the two processes roughly offset each other.

In order to see to what extent this is true, the calculations in table II were made. From the per cent. of alcohol-insoluble residue was subtracted its protein (N x 6.25) and starch. The remainder is termed "polysaccharide" for convenience, although there are no doubt small amounts of fat and other substances present. It will be seen that in the first two stages

[1] The nitrogen in the 80 per cent. alcohol-insoluble residue is usually called the "protein nitrogen," although use of this term is not free from objections.

TABLE I

Changes in composition of whole peas kept in open beakers at 25°C. (All analyses on basis of 100 gm. of original fresh material)

No.	Age from blossom	Time after picking	Dry matter	Weight of 100 gm. original peas	Alcohol-insoluble residue	Reducing sugars	Sucrose	Total sugars	Starch	Soluble N	Insoluble N	Total N	Starch / Sugars	Insoluble N / Soluble N	Total analyzed constituents[*]
	days	days	per cent.	gm.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.			per cent.
0	2	0	15.3	100.0	8.03	0.75	1.89	2.64	0.0	0.56	0.50	1.06	0.0	0.90	14.23
1	6	0	18.2	100.0	7.22	0.50	7.10	7.60	0.91	0.36	0.41	0.77	0.11	1.13	17.07
2		4		95.0	8.87	0.30	4.00	4.30	0.72	0.35	0.43	0.78	0.17	1.20	15.36
3		1		83.8	10.86	0.50	2.33	2.83	0.94	0.36	0.45	0.81	0.32	1.27	15.94
6		7		15.8	10.94	0.09	1.90	1.99	0.29	0.18	0.61	0.79	0.15	3.47	14.06
8		50		14.8	11.14	0.45	0.80	1.25	0.73	0.22	0.60	0.82	0.92	2.80	13.76
9	10	0	19.7	100.0	9.66	0.44	7.23	7.67	1.61	0.26	0.53	0.79	0.21	2.08	18.95
10 (ripe for cannery)		4		93.4	10.33	0.32	4.57	4.89	1.00	0.28	0.53	0.81	0.21	1.89	16.97
11		1		77.8	11.80	0.30	3.17	3.47	0.96	0.26	0.58	0.84	0.28	2.23	16.89
12		2		55.2	11.87	0.45	1.33	1.78	1.03	0.33	0.49	0.82	0.58	1.51	15.71
13		4		34.1	11.65	0.87	1.17	2.04	0.65	0.44	0.45	0.89	0.32	1.03	16.44
23	17	0	33.1	100.0	23.25	0.17	2.93	3.10	3.37	0.11	1.30	1.41	1.09	11.66	27.04
24		1		72.5	27.58	0.11	1.47	1.58	3.22	0.11	1.29	1.40	2.03	11.47	29.84
26		4		43.7	28.12	0.07	1.13	1.20	3.58	0.09	1.36	1.45	0.29	15.88	29.88
27		46		36.3	26.80	0.31	1.03	1.34	3.35	0.11	1.30	1.41	0.25	12.07	28.83

[*] = alcohol-insoluble residue + total sugars + (sol. N x 6.25).
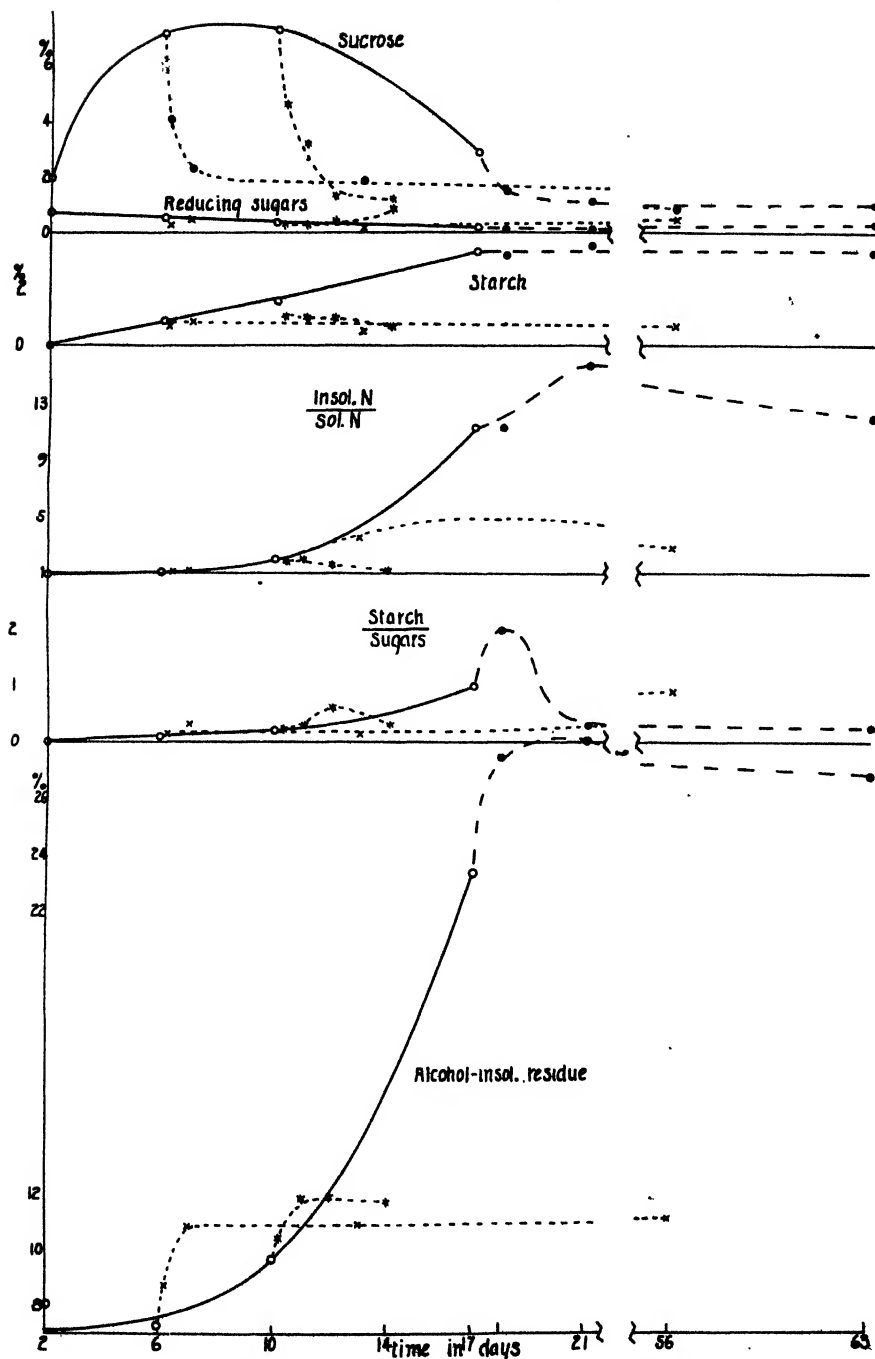
FIG. 1. Changes in the composition of whole peas after removal from the vines. Calculated on basis of 100 gm. of fresh material.

TABLE II

RELATION BETWEEN SUCROSE AND "POLYSACCHARIDE" IN PEAS AFTER REMOVAL
FROM THE VINES

No.	AGE FROM BLOS- SOMS	TIME AFTER PICK- ING	A ALCOHOL- INSOLU- BLE RESIDUE	B POLY- SACCHA- RIDE*	C INCREASE IN POLY- SACCHA- RIDE	D DECREASE IN SUCROSE	E 100.$\frac{C}{D}$
	days	*days*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	
1	6	0	7.22	3.75	....	....	....
2		¼	8.87	5.46	1.71	3.10	55
3		1	10.86	7.11	3.36	4.77	70
6		7	10.94	6.84	3.09	5.20	60
9	10	0	9.66	4.74	....	....	....
10		¼	10.33	6.02	1.28	2.66	46
11		1	11.80	7.22	2.48	4.06	59
12		2	11.87	7.78	3.04	5.90	51
13		4	11.65	8.18	3.44	6.06	61
23	17	0	23.25	11.76	....	....	....
24		1	27.58	16.30	4.54	1.46	310
26		4	28.12	16.04	4.28	1.80	240

* "Polysaccharide" = Alcohol-insoluble residue – (protein + starch).

the decrease in sucrose can account for a large part of the increase in the
"polysaccharide" phase of the residue. In the last stage it cannot begin
to account for it since the increase in "polysaccharide" is three or four
times as great as the decrease in sucrose. We have no explanation to offer
for this. Of course, we are merely surmising that the sucrose is converted
into the "polysaccharide," since there is no direct proof for it.

The chief significance in these data is the fact that sucrose is lost so
quickly after the peas are picked. This partly explains rapid deterioration
of quality in peas.

In order to show the changes in composition of peas during growth,
part of the above data were calculated on a dry matter basis and assembled
in table III. These are now directly comparable with BOSWELL'S
tables (4).

In the column headed "weight of 100 gm. of original peas" it will be
seen that by the seventh day after picking the weight is less than that of
the dry matter present originally. The moisture content of the stored
samples could not be determined because of lack of material. This loss in
material is no doubt due to respiration, and probably can account for that
portion of the sucrose which was not converted into "polysaccharide."

TABLE III

CHANGES IN CHEMICAL COMPOSITION OF PEAS ON THE VINES. VARIETY: "ROGERS C." DRY MATTER BASIS. SAMPLES TAKEN IMMEDIATELY AFTER PICKING

No.	AGE FROM BLOSSOM	DRY MATTER	ALCOHOL-INSOLUBLE RESIDUE	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	SOLUBLE N	INSOLUBLE N	TOTAL N	STARCH/SUGARS	INSOLUBLE N/SOLUBLE N
	days	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.		
0	2	15.3	52.60	4.90	12.36	17.26	0.00	3.66	3.27	6.93	0.00	0.90
1	6	18.2	39.68	2.75	39.00	41.75	5.00	1.98	2.25	4.23	0.11	1.13
9	10	19.7	49.05	2.23	36.85	39.08	8.17	1.32	2.69	4.01	0.21	2.04
23	17	33.1	70.21	0.51	8.85	9.36	10.18	0.33	3.93	4.26	1.09	11.86

Chemical changes in macerated peas

In order to obtain some idea of the enzymes which are active in peas, the composition was determined of some samples that had been ground and had been standing under water for various lengths of time in the presence of toluene. These samples had been taken in the manner described above, and were of the same size, 30 gm. They were ground at once and covered with 100 ml. of water and 3 per cent. of toluene. After the samples had stood for stated intervals they were made up with 95 per cent. alcohol to 80 per cent. of alcohol, placed in the extractors and extracted as has been described for the other samples. The data are presented in table IV and in figure 2.

From the chart we see that the sucrose and reducing sugars are in striking contrast to their behavior in the whole peas. In the macerated peas the decrease in sucrose is accompanied by an increase in reducing sugars. This means, no doubt, that sucrose is very active. In the same way, the starch decreases, indicating the action of diastase. The protein-nonprotein ratio also decreases, which must mean that proteases are functioning. The alcohol-insoluble residue shows no appreciable change in the first set of samples, and only a slight increase in the second.

In table V are presented calculations on the relation between the changes in sugars and in "polysaccharide" in these samples. The calculations are similar to those in table II for whole peas, except that in the present case the content of total sugars was considered instead of that of sucrose. The results are irregular in trying to account for the decrease in sugars by a concomitant increase in "polysaccharide," but they indicate in the first set of samples that the lost sugars can roughly account for the "polysaccharide." In the second set, the lost sugars can account for only from one-fourth to two-thirds of it. Again it is not apparent what materials do go into the formation of the "polysaccharide."

The last column in tables I and III contains the sum of all analyzed constituents. In the 6 and 10 day samples in both cases there is a marked drop in these constituents during the first few hours. This must represent loss of material by respiration, even in the macerated material.

In the 17-day samples, however, there is an increase in total analyzed material during the first few hours. This can only mean that in the fresh material there is some constituent soluble in 80 per cent. alcohol which is not accounted for in the analysis, but which later is converted into some material which is accounted for. No suggestion is offered as to what this material may be.

BROWN (5) made similar analyses of peas which had been stored in various kinds of paper wrappers. He states his results as follows: "Peas

TABLE IV

CHANGES IN COMPOSITION OF MACERATED PEAS KEPT AT 25°C.   FRESH WEIGHT BASIS

No.	AGE FROM BLOSSOM	TIME AFTER MACERATION	DRY MATTER	ALCOHOL-INSOLUBLE RESIDUE	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	SOLUBLE N	INSOLUBLE N	TOTAL N	STARCH SUGARS	INSOLUBLE N SOLUBLE N	TOTAL ANALYZED CONSTITUENTS*
	days	hours	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.			
9 (ripe for canning)	10	0	19.7	9.66	0.44	7.23	7.67	1.61	0.26	0.53	0.79	0.21	2.08	18.95
14		3	......	9.96	0.27	5.00	5.27	0.78	0.34	......	......	0.15	......	17.35
16		24	......	10.53	1.03	4.27	5.30	0.63	0.38	0.42	0.80	0.12	1.10	18.20
17		48	......	8.76	2.42	2.17	4.59	0.76	0.48	0.28	0.76	0.16	0.58	16.35
18		96	......	8.73	5.49	1.10	6.59	0.62	0.51	0.27	0.78	0.09	0.53	18.51
23	17	0	33.1	23.25	0.17	2.93	3.10	3.37	0.11	1.30	1.41	1.09	11.66	27.04
28		24	......	24.60	0.56	1.43	1.99	2.74	0.34	1.02	1.36	1.37	3.01	28.72
29		48	......	25.60	0.75	0.83	1.38	2.92	0.38	1.03	1.41	2.11	2.70	29.36
30		96	......	25.40	1.20	0.60	1.80	1.21	0.45	0.97	1.64	0.67	2.16	30.01

* = Alcohol-insoluble residue + total sugars + (sol. N x 6.25).
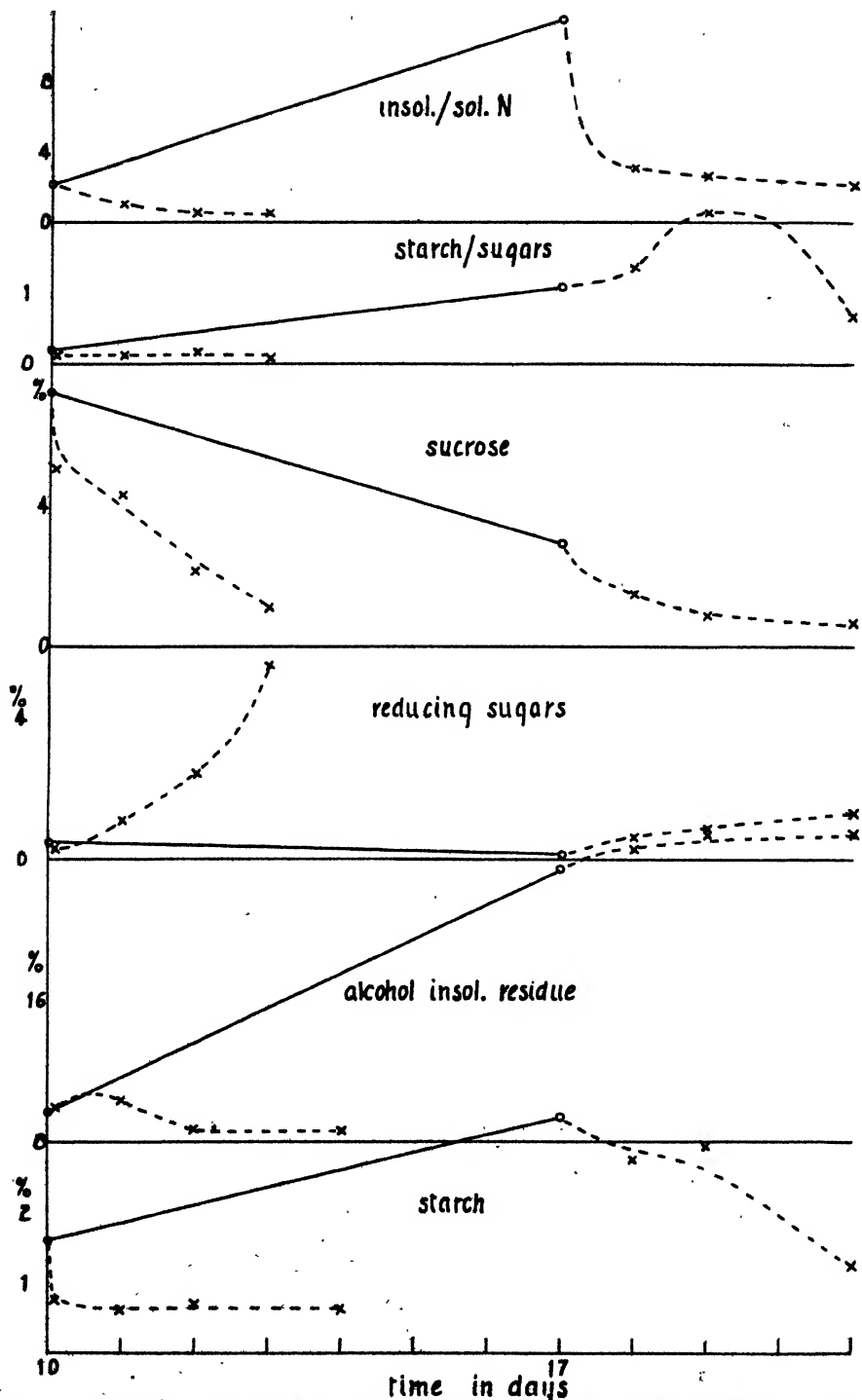
FIG. 2.   Changes in the composition of macerated peas.   Calculated on basis of 100 gm.
of fresh material.

TABLE V

RELATION BETWEEN TOTAL SUGARS AND "POLYSACCHARIDE" IN PEAS AFTER MACERATION

No.	Age from blossoms	Time after picking	A Alcohol-insoluble residue	B Poly-saccharide*	C Increase in poly-saccharide	D Decrease in total sugars	E $100.\dfrac{C}{D}$
	days	days	per cent.	per cent.	per cent.	per cent.	
9	10	0	9.66	4.74	....	....	....
14		½	9.96	....	....	....	....
16		1	10.53	7.27	2.53	2.37	107
17		2	8.76	6.25	1.51	3.08	49
18		4	8.73	6.42	1.68	1.08	155
23	17	0	23.25	11.75	....	....	....
28		1	24.60	15.48	3.73	1.11	336
29		2	25.60	14.24	2.49	1.72	145
30		4	25.40	18.11	6.36	1.30	490

* "Polysaccharide" = Alcohol-insoluble residue – (protein + starch).

which contained 18.6 per cent. sugar (as invert), at the start contained only 2.1 to 2.8 per cent. at the end of seven days storage at high temperatures, while those held at 32° F. still possessed most of their original sugar (60–80 per cent.). Apparently the sugar was first converted into acid hydrolyzable material, as is shown by the cold storage lots where only a partial sugar loss is shown, and later to starch, as is shown by the large amount in the high temperature lots, where the starch content has increased 400 per cent., though the acid-hydrolyzable material is but little greater than in the cold storage lots. The dry matter content, of course, varies with the storage conditions."

Thus his results and conclusions coincide very well with the present work, although here the "polysaccharide" was assumed to exist, and was not determined by acid hydrolysis.

It is obvious that both in whole and in macerated peas hydrolysis of carbohydrates and respiration are going on simultaneously. In the whole peas respiration predominates, and reducing sugars do not accumulate. In the macerated, hydrolysis predominates, and reducing sugars do accumulate. These data do not answer the question whether sucrose can be respired directly, or whether it must first be hydrolyzed.

The amount of 80 per cent. alcohol-insoluble residue increases as the peas are kept. In the immature peas (picked six days after blossoming) this increase during the first twenty-four hours is 50 per cent., in the ripe

TABLE VI

EFFECT OF FREEZING ON CHEMICAL COMPOSITION OF PEAS AT CANNING STAGE

FRESH WEIGHT BASIS

TIME AFTER PICKING	TEMPERATURE*	ALCOHOL-IN-SOLUBLE RESIDUE	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	SOLUBLE N	INSOLUBLE N	TOTAL N	STARCH / SUGARS	INSOLUBLE N / SOLUBLE N	TOTAL ANALYZED MATERIAL
hours	deg. C.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.			per cent.
0	--	9.66	0.44	7.23	7.67	1.61	0.26	0.53	0.79	0.21	2.08	18.95
24	-20	11.20	0.44	4.57	5.01	1.46	0.31	0.55	0.86	0.29	1.80	18.15
24	+25	11.80	0.30	3.17	3.47	0.96	0.26	0.58	0.84	0.28	2.23	16.89

* Treatment.

-- = Analyzed immediately.

-20 = 24 hrs. at -20° C.

+25 = 24 hrs. at +25° C.

peas (picked ten days after blossoming) it is 22, and in the overripe peas (picked seventeen days after blossoming) it is 18.5 per cent. In the macerated samples this residue increases at first and decreases later. The present experiments do not offer any explanation for this phenomenon.

Effect of freezing on composition of peas

It is reasonable to suppose that the rate of respiration will be lower at lower temperatures. To test this a sample of the peas picked ten days after blossoming was kept in a freezing room at a temperature of –20° C. for twenty-four hours. The results of the determinations made upon these peas are presented in table VI. For the purposes of comparison the results of the determinations upon these and upon a similar lot freshly picked are set side by side.

The low temperature has not checked the respiration as much as might have been expected. This is probably explainable by the slow cooling of the samples in the freezing room or to the very intensive respiration during the time required for the operations of sampling. The effect of low temperatures on the chemical changes in peas requires further study.

Conclusions

Definite changes in the chemical composition of shelled peas begin to take place immediately. The most important and striking of these is the decrease in the sucrose content. This partly explains the change in the flavor. The percentage of alcohol-insoluble residue increases, possibly at the expense of sucrose.

The changes in the composition of the peas which are presumably related to the respiration are interrupted when the peas become dry.

When freshly picked peas are macerated and kept under toluene-water, sucrose and starch decrease, reducing sugars increase, and non-protein N increases. This indicates that sucrase, diatase, and protease are present and active.

Even when freshly picked peas are placed at – 20° C. for 24 hours there is marked evidence of change in composition, probably because of rapid changes during the brief period before freezing actually takes place.

These experiments show in definite terms the necessity of cooking or canning peas immediately after shelling.

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LITERATURE CITED

1. BENOY, MARJORIE P. The respiration factor in the deterioration of fresh vegetables at room temperature. Jour. Agr. Res. 39: 75–80. 1929.

2. BLACKMAN, F. F., and PARIJA, P. Analytic studies in plant respiration. I.—The respiration of a population of senescent ripening apples. Proc. Roy. Soc. London B **103**: 412–445. 1928.

3. BODNÁR, J., and HOFFNER, P. Beiträge zur biochemischen Kenntnis der postmortalen Pflanzenatmung. Biochem. Zeitschr. **165**: 145–167. 1925.

4. BOSWELL, VICTOR R. Factors influencing yield and quality of peas. Maryland Univ. Bull. 306. 1929.

5. BROWN, H. C. Paper wrappers and their effect upon physical and chemical properties of horticultural products. Michigan Agr. Exp. Sta. Techn. Bull. 87. 1928.

6. GOWEN, P. L. Effect on quality of holding shelled peas. The Canner, Convention no. p. 112. 1928.

7. —————. The blanching of peas. The Canner, Convention no. p. 100. 1929.

8. HAAS, P., and HILL, T. G. The chemistry of plant products. 1929.

9. IWANOFF, N. N., ALEXANDROWA, R. S., and KUDRJAWZEWA, M. A. Über die Umwandlung der Zuckerarten beim Reifen der Früchte von Wassermelonen. Biochem. Zeitschr. **212**: 267–279. 1929.

10. LASAUSSE, E., GUERITHAULT, B., and PELLERIN. Maturing of peas. [Translated title]. Bull. sci. Pharmacol. **35**: 337–345. 1928. Chem. Abstr. **22**: 3193. 1928.

11. LÜNING, O., and BEYER, E. Über den Nachweis nachgemachter konservierter Erbsen. Zeitschr. Unters. Lebensm. **57**: 76–85. 1929.

12. ROSA, J. T. Changes in composition during ripening and storage of melons. Hilgardia **3**: 421–443. 1928.

13. WALKER, D. The production and marketing of New York market peas. Cornell Univ. Bull. 475. 1929.

A METHOD FOR THE RAPID DETERMINATION OF PHOSPHATE IN FRESH PLANT TISSUES[1]

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Little work has been done on the distribution of phosphate phosphorus in living plants. The colorimetric method worked out by FISKE and SUB-BAROW (2) for phosphate in animal substances should be equally good when applied to an appropriate solution from plant tissues. Preliminary trials showed that the solution of plant tissue must not be made alkaline, since phosphate was precipitated in alkaline solution. However, satisfactory solutions were obtained by using a weak acid solution and a suitable quantity of powdered charcoal or carbon black. The procedure and results follow.

Preparation of standard solution and checking of reagents

Make up a standard solution by dissolving 0.1755 gm. potassium dihydrogen phosphate in a liter of water. Five cc. of this solution contains 0.2 mg. of phosphate phosphorus. Add the same amount of acid to it as in the unknown, and neutralize just as is done in the procedure. A blank should also be prepared to check the reagents, and one standard should be treated with 1 per cent. acid and charcoal just as the unknown to test for absorption of phosphate by the charcoal, since different grades vary in their absorptive power (2). If the charcoal being used causes absorption, a grade should be obtained which does not. If the charcoal contains phosphate phosphorus it should be digested with 5 per cent. by volume sulphuric acid for one-half hour and leached with 1 per cent. by volume sulphuric acid until the leachings give no test for phosphates by the FISKE and SUBBAROW method, as directed below.

Procedure

Triturate thoroughly enough green plant tissue to give 0.1 to 0.3 mg. phosphate phosphorus (usually 1 gram of tissue), in a mortar, with 5 gm. of fine "decolorizing charcoal."[2] Now add exactly 50 cc. of 1 per cent. by volume sulphuric acid and mix well with the black paste. Allow to stand 5 minutes and filter. If not clear and colorless, treatment with more charcoal will be needed. The amount of charcoal used in trituration should be regulated according to the amount required to give a clear solution. Three to five grams was found to be sufficient for a one-gram sample. When the

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Decolorizing charcoal from J. T. Baker Chemical Co. was used.

solution is cleared, add a few drops of phenolphthalein to an aliquot (usually 25 cc.) and bring just to neutrality with dilute sodium hydroxide.

Make the neutral solutions of the unknown, blank, and standard up to about 70 cc. and add 10 cc. of 2.5 per cent. ammonium molybdate made up in 5 N sulphuric acid. Mix and add 4 cc. of 1, 2, 4-amino-naphtholsulphonic acid, prepared as directed by FISKE and SUBBAROW (1). Mix and make up to 100 cc. After ten minutes read in a colorimeter and calculate the phosphate phosphorus in the original plant tissues.

Results

Table I shows excellent duplication. The duplicates were taken from

TABLE I

PHOSPHATE PHOSPHORUS IN GREEN PLANT TISSUES

(1-GRAM SAMPLES WERE TREATED WITH 5 GRAMS OF PHOSPHATE-FREE CHARCOAL AND 1 PER CENT. SULPHURIC ACID)

TISSUE	SAMPLE NO.	DET. 1	DET. 2	AVERAGE	ERROR FROM AVERAGE
		ppm.	ppm.	ppm.	per cent.
Tomato	1	480	488	484	0.8
Tobacco	1	109	109	109	0
Lettuce	1	131	131	131	0
"	2	344	344	344	0
"	3	296	280	288	2.8
"	4	342	342	342	0
"	8	448	448	448	0
"	11	416	400	408	2.0
"	17	704	672	688	2.3
"	18	448	448	448	0

the same leaf by selecting a large leaf and using tissue between the large veins. In this way uniform samples were obtained. The different lettuce samples were from plots which varied greatly in treatment. No. 17 had large applications of 85 per cent. syrupy phosphoric acid which explains its high phosphate phosphorus content. No. 3 was from a plot high in lime which probably explains the smaller amount of phosphate phosphorus obtained. No. 1 was a fresh compost not comparable with the soils in the other plots.

Table II shows conclusively that the charcoal used did not absorb phosphate from the acid solutions used.

Choice of reducing agent

TRUOG and MEYER (3) used the method of DENIGÈS in which stannous chloride is employed as a reducing agent. Their tests, however, show that

TABLE II

Recovery of phosphate in acid solutions filtered from phosphate-free charcoal (Treated with the same reagents as were used on the plant tissue)

TISSUE	P ADDED	P RECOVERED	ERROR FROM AVERAGE
	mg.	mg.	per cent.
1	0.185	0.185	0
2	0.185	0.185	0
3	0.185	0.185	0
4	0.037	0.037	0
5	0.370	0.370	0

the range of acidity and concentration of molybdate in which accurate results are obtained, is very narrow. If the acidity is weak the molybdate gives a color in the absence of phosphate; and if it is too strong, the blue color fails to form even if phosphate is present. It is also difficult to maintain a uniform solution of stannous chloride, a layer of mineral oil being recommended to preserve it. The solution of 1, 2, 4-amino-naphthol-sulphonic acid will keep at least a month without any special treatment. It does not have such a narrow range of acidity in which it is accurate and never causes a color to form when phosphorus is not present, as long as the solution is acid and cold. On boiling, however, a blue color is produced without phosphorus. Trials with different degrees of acidity, from neutral to very acid, in the cold, produced no color from molybdate alone. Stannous chloride acts on molybdate in the cold even if phosphate is not present, unless a certain acidity is maintained, and small increases in temperature may cause a color even if the acidity is right.

Another advantage of the amino-naphtholsulphonic acid reagent is that it is not so sensitive to interference by the presence of ferric iron as stannous chloride. TROUG and MEYER (3) found that the limit with stannous chloride was 2 ppm. of ferric iron. Table III shows that as much as 30 ppm. of ferric iron may be present in the solution with 1, 2, 4-amino-naphtholsulphonic acid without interfering.

It is true that stannous chloride brings the color faster than the amino-naphtholsulphonic acid reagent, but as long as the standard and unknowns are treated alike as to time and reagents accurate results are obtained as shown by tables I and II. Interference by silica was not a factor in the plant solutions, since silica would not be put into solution by the weak acid used, even if the plant contained a significant amount.

TABLE III
EFFECT OF FERRIC IRON ON THE BLUE PHOSPHATE COLOR
(FERRIC SULPHATE WAS USED)

FE	P ADDED	P RECOVERED	NOTES ON COLOR
ppm.	mg.	mg.	
5	0.20	0.225	Blue matched well
5	0.20	0.20	" " "
10	0.20	0.20	" " "
10	0.20	0.20	" " "
20	0.20	0.20	" " "
30	0.20	0.20	Blue, slight cloudiness
40	0.20	0.20	Blue, cloudy
50	0.20	0.12	Slight green, quite cloudy
60	0.20		Greenish
70	0.20		Very green

Titanium and arsenates cause errors if present (3), but would not likely be present in significant amounts in plant extracts. TRUOG and MEYER (3) also found that nitrate, if present at the rate of 200 ppm. of nitrogen, caused a fading of the color. However, it would be highly improbable that a one-gram sample of any plant in 100 cc. would cause a concentration of 200 ppm. of nitrate.

Summary
1. A method of determining phosphate phosphorus in fresh plant tissue is outlined. The tissue is extracted with dilute sulphuric acid and cleared with phosphate-free powdered charcoal or carbon black. The method of FISKE and SUBBAROW is used on the cleared extract.

2. Data are presented which show good duplicates on tomato, tobacco and lettuce leaves.

3. No adsorption of phosphate by the charcoal used could be detected.

4. Preference is given to 1, 2, 4-amino-naphtholsulphonic acid over stannous chloride as a reducing agent, because:

(1) The reagent keeps better.

(2) It does not require as narrow a range of acidity for accuracy.

(3) A blue color does not develop in the absence of phosphate at any degree of acidity used in these experiments.

(4) Larger quantities of iron may be present in the tissue extract without causing interference.

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LITERATURE CITED

1. FISKE, C. H., and SUBBAROW, Y. The colorimetric determination of phosphorus. Jour. Biol. Chem. **63**: 375–400. 1925.
2. HARPER, H. J. The accurate determination of nitrates in soils. Jour. Ind. Eng. Chem. **16**: 180–183. 1924.
3. TRUOG, E., and MEYER, A. H. Improvements in the Denigès colorimetric method for phosphorus and arsenic. Jour. Ind. Eng. Chem. (Analytical edition) **1**: 136–139. 1929.

THE ASKENASY DEMONSTRATION

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In the third edition of Palladin's Plant Physiology (pp. 147–150, 1926), LIVINGSTON calls attention to the fundamental and far-reaching importance of liquid tension in plants, and laments the fact that it is rarely demonstrated in university or college courses in either physics or plant physiology. There is contained in this textbook a brief account of the precautions that seem to be necessary in order to assure the success of the ASKENASY demonstration. This was followed by a paper[1] giving additional information on a simplified technique, together with a request that other experimenters record their successes or failures with the demonstration.

The writer has experimented off and on with the ASKENASY demonstration during several years. It is not only fascinating to try to better former traction records, but trial after trial leads one to better comprehension of the various forces operating and to appreciate more fully the phenomena existing in the living plant. Various procedures have been tried out and their effects have been carefully noted. Different membranes have been used, in the attempt to find one more nearly comparable with the natural plant membrane. While the experiments leave much to be desired, they have been attended with sufficient success and with such regularity that a traction experiment has been made a regular demonstration in the course in plant physiology at this university, and the apparatus is kept set up so that it may be used from year to year.

The first apparatus was set up in the manner illustrated in PALLADIN, using a single five-foot length of glass tubing with a one-millimeter capillary, a standard thick-walled atmometer cup, and a filter pump of twenty-one pounds capacity for suction. As typical of results one is likely to obtain at first, eleven trials gave the following in centimeters of rise of mercury column above mercury level in lower reservoir: 30.5, 81.3, 80.0, 63.8, failure, 100.3, 100.0, 107.5, 99.7, 103.6, failure. Uncorrected barometric pressure for this series varied between 72.9 cm. and 74.1 cm. It should be further noted that in the sixth trial the meniscus in the cup apparently broke at 95.2 cm., the level fell about 2 mm., and then started back, giving a total rise of 100.3 cm.; and again, in the eighth trial the column fell over 1 cm. at 103.5 cm., followed by recovery, and making a final height of 107.5 cm. In the many experiments subsequently performed, this phenomenon was observed on only one other occasion. The cause has been referred to as a breaking of one or more menisci, but since the cup is opaque and anything

[1] LIVINGSTON, B. E. and LUBIN, GRACE. The ASKENASY demonstration of traction transmitted through liquid water. Science n.s. 65: 376–379. 1927.

taking place in it is invisible, the phenomenon is a matter for conjecture; and some slight readjustment of parts, magnified by the small bore of the capillary, may be the explanation. The dropping of the mercury column was sudden, and if it was due to breaking of the meniscus, the method of recovery is a matter of considerable importance; for, as pointed out by LIVINGSTON, the water system usually breaks, and the mercury column then falls quickly to a height corresponding with the current reading of the barometer. LIVINGSTON further states that the system breaks, sometimes in the water in capillary or cup, and sometimes in the mercury. In our experiments, always using distilled water, but often exposing it in the boiling cup to the atmosphere for days before a trial was made, breaking of the system was never observed to take place (in the trials which were at all successful) save in the porous cup.

As the desirability of a longer capillary tube became apparent, the idea of joining two lengths of tubing together was suggested. Various kinds of bonds were tried, all without success. Finally, a method was perfected for fusing two glass tubes together, using a blow pipe and infinite patience, since it is easy to draw the capillary. The operation has been successfully applied on several subsequent occasions, but it is not recommended to the average glass blower. Later, it has been learned that glass tubing may be obtained in longer lengths on special order from the manufacturer. This would be more satisfactory, but might entail some months of waiting. Hard glass would probably be better than soda-lime glass. One set-up, made from soft glass, gave a great deal of trouble due to numerous minute holes developing successively in the upper bend of the tube after a short period of use.

The construction of a suitable evaporation membrane has been the subject of much thought and experimentation. The porous cup constitutes an artificial leaf, just as the capillary tube takes the place of a single vessel. In a living leaf the place where water loss takes place is not essentially through the stomata, but internally from the moist surfaces of the cells which abut upon the intercellular spaces back of the stomata. The walls of these cells and the plasma membranes within are not the porous structures that clay cups are. Ideally, the nearer the porous cup approximates the physical conditions of the evaporating mechanism of the living leaf, the more successful should be the demonstration as measured in lifting-power. The difference in behavior of a larger-pored cup as contrasted with a finer-pored condition (meaning large menisci as against small menisci) was demonstrated, as follows. A thin-walled unglazed porcelain cup was selected, which was boiled in sodium hydroxide and carefully washed; the rubber stopper was new and also properly treated to remove air and sulphur. Five separate trials with this cup gave three failures and the maximum rise ob-

tained was 29.2 cm. of mercury. The cup was then removed and a copper-ferrocyanide membrane was deposited in the walls by the ordinary procedure. Seven trials with the treated cup gave uncorrected results in centimeters as follows: 91.7, 96.0, 91.7, 91.0, 80.0, 88.2, and 58.0, the barometric pressure varying from 72.5 cm. to 75.0 cm. Similar thin-walled cups were used, but impregnated with a very dilute solution of cellulose acetate (Cellon, Paris) in acetone. Results were as follows, in centimeters of mercury column: 57.0, 62.0, 79.6, 63.5, 75.0, 81.0, 54.0, 57.0, 57.5, 57.7, and 82.2. A cellulose acetate membrane presents at least two difficulties; water evaporates very slowly from the treated cup; but more important, it is very difficult to free the system from imprisoned air, and perhaps impossible entirely to rid the system of the smaller bubbles.

So much success attended the trials with a copper-ferrocyanide precipitation membrane that the idea of making a hot-precipitation membrane was conceived. It is obvious that when heated a porous cup is expanded and the pores are larger than in the cool state. If a cup is then boiled in distilled water with suction to remove as much air as possible and is then filled with hot copper sulphate (2.5 gm. per liter) and suspended in a beaker of hot potassium ferrocyanide (2.1 gm. per liter), the whole being maintained in a hot-water bath for several hours, upon cooling the precipitated membrane must undergo a reorganization which would seemingly tend to make it more compact. Many trials with such a membrane seem to indicate that such is the case. Such a membrane is quite permeable with suction during the preliminary boiling to free the system from air, and water comes down the tube freely. On cooling, however, and making a trial test, such a membrane may be found to be quite impermeable. Enough experimentation has been done to indicate that there is a happy medium as to the amount of heat to apply during impregnation, and good results have attended a moderate application of heat during the process of precipitation. The method of hot-precipitation is worthy of further investigation.

Much time has been spent in trying to arrive at a more effective method of removing air from the system than is possible by the simple expedient of boiling and suction, especially in the case of using rather impermeable porous cups or membranes. Two-holed rubber stoppers were tried, a stop-cock, with and without funnel attached, being inserted in the second hole. Filling the funnel and tube with boiling water, it was thought that the system might thus be flushed out, the gas bubbles being forced out at the lower end of the capillary. This idea was soon abandoned. More trouble was experienced from gas bubbles than before. Solid glass rods were inserted in the hole. These could be withdrawn under water and reinserted. No great success was attained. Either the inrushing water column was sheared off at the instant of closing, thus creating a vapor

tension area which does not "heal," or the forcing in of the rod disengaged
gas bubbles from the interior of the stopper, which were forced out by the
compression. Metal pistons were also similarly inserted, by which means
a pumping action could be set up, combined with agitation. Their chief
effect was to loosen the stopper. On the other hand, several devices, men-
tioned presently, were more successful.

In all, about a hundred and twenty trials have been conducted, many
with considerable success, and some were frankly failures. The best record
obtained was made with a LIVINGSTON calibrated atmometer cup (no. 5–548,
fifteen ×), boiled in weak NaOH and washed well, but otherwise untreated.
The capillary tube was constructed from two fused, five-foot lengths of
tubing, the upper end of which was bent into a U-shape, three and one-half
inches high and four inches across, since it has been found that gas bubbles
are more easily removed from gradual bends than from sharp angles. Pre-
liminary to the fourth trial, the apparatus was boiled up for a half hour,
with several periods during which the suction was released in the manner
suggested by LIVINGSTON. The apparatus was allowed to cool, and on the
next day full suction was applied, which was successfully withstood. The
boiling pot was then heated to 60° C., and then quickly removed. Seven-
teen minutes afterward the column fell after reaching an uncorrected
height of 150.0 cm., the barometer reading at the time being 73.9 cm. This
indicates a pressure of more than two atmospheres. This same cup also
gave other good records.

For maximum success with the ASKENASY demonstration, in addition
to the precautions already suggested by LIVINGSTON, the following sugges-
tions are made:

a. The mercury used in the lower reservoir should be pure and clean.
As soon as it becomes glazed on top, it should be removed and washed
before further use.

b. It is an advantage to have the end of the capillary tube relatively
close to the surface of the mercury. If, in addition, the end of the tube
is beveled up to the capillary, using a file and water lubricant, the removal
of air is facilitated, especially in the case of less permeable porous cups
and membranes.

c. The rubber stopper must fit tightly in the cup. This is best accom-
plished by first twisting it in, all parts being bathed with water. After
this the stopper may be further inserted by a combined kneading in of the
edges at the same time that a pushing thrust is used. When the stopper
is well-seated, it may be slipped onto the upper end of the capillary tube,
which should have been previously marked with a thread gauge to bring the
end of the tube flush with the under side of the stopper. It is perhaps an

advantage previously to prepare the lower end of the stopper by cupping it with a sharp safety razor blade into a low cone, which gives little chance for air bubbles to lodge under the stopper.

d. There is no advantage, when using the ordinary porous cup, in long continued boiling, although this may not be true with treated cups which are difficult to free from confined air.

e. In making class-room demonstrations, it is best to try out the apparatus first by application of full suction, gradually applied and as gradually released. If the system does not break, the experiment is likely to be successful. Preliminary heating of the boiling pot to 60°–65° C. before beginning a trial allows the teacher to finish the demonstration in a few minutes' time, and the rise of the mercury column is so rapid that it is eagerly followed by the student. A convenient marker to record the progress made by the mercury is made from a short piece of rubber tubing, split, and slipped over the capillary tube. A little water between the surfaces in contact will furnish lubrication so that it may easily be moved. Jarring of the tube should be avoided, and may be largely prevented with proper rigid supports and ordinary care, not neglecting provision for expansion and contraction of parts.

This is a brief record of progress and difficulties. However, no teacher should be easily discouraged. With a suitable porous cup and ordinary laboratory facilities, the ASKENASY demonstration is not at all difficult to make; and it is hoped that it may be more generally employed in course work.

UNIVERSITY OF WISCONSIN,
    MADISON, WISCONSIN.

NICOLAS THÉODORE DE SAUSSURE
1767–1845

BRIEF PAPERS

NICOLAS THÉODORE DE SAUSSURE[1]

(WITH ONE PLATE AND THREE FIGURES)

NICOLAS THÉODORE DE SAUSSURE (plate IV) adopted quantitative methods in dealing with the problem of plant nutrition at a time when practically all other botanists were concerning themselves with theories of the vital forces which supplied food to plants in the form of the mysterious substance known as humus. DE SAUSSURE sustained and added to the discoveries of INGEN-HOUSZ and SENEBIER and proved that the green parts of plants take up and decompose a substance of the air at the same time that they assimilate water. His experiments on carbon assimilation are numerous and precise, and were presented with great perspicuity in his "Recherches Chimiques sur la Végétation" published in 1804. DE SAUSSURE found that the decomposition of carbon dioxide by green plant parts was a process necessary for the continued life and growth of the plant, but in addition he found that the carbon dioxide content of the atmosphere surrounding a plant might be increased, by artificial means, up to the point where it actually was injurious to the plant. Large quantities of carbon dioxide in the air were favorable adjuncts only if the plants were in condition to assimilate rapidly. After determining the amount of carbon appropriated by a plant, DE SAUSSURE found that the plant increased in weight by an amount out of proportion to the quantity of carbon fixed. Consequently, although he was of the opinion that the great mass of the vegetative body was built up from the components of the atmosphere, it was clear that a part of the vegetable mass was derived from the fixation or utilization of the soil solution.

The family of DE SAUSSURE was of French origin but about the middle of the sixteenth century they were among the religious refugees who fled from France to the little Republic of Geneva. Members of the family became prominent in the governmental affairs of the republic, and even at the present time some of its members are citizens of Geneva. The DE SAUSSURES have been interested in scientific pursuits as well as in the course of government. NICOLAS, the grandfather of NICOLAS THÉODORE, was a country gentleman engaged in the management of his various estates and interested in agricultural matters. He published several practical treatises; on methods of cultivation, pruning, crop failures, and soil fertility. His son, HORACE BENEDICT, who was the father of NICOLAS THÉODORE, was a scientist also

and we are indebted to him for many of the data on which the very foundations of geology rest. HORACE BENEDICT DE SAUSSURE, figure 1, was one of the first and most successful Alpine travellers: perhaps he was also one of

FIG. 1. HORACE BENEDICT DE SAUSSURE, father of NICOLAS THÉODORE DE SAUSSURE

the most unique, for he undertook his journeys for the sake of studying geology, physics, and other natural phenomena as well as for pleasure. Mountain chains, rocks, streams, glaciers, and the atmosphere were studied so diligently that even today a large share of his work remains undisputed. He must have been ingenious as well as diligent, for he invented or perfected a number of instruments which were required for the physical measurements in his work. With such a background and such an environment it is not surprising that NICOLAS THÉODORE turned his thoughts to scientific studies. Much of the life of NICOLAS THÉODORE DE SAUSSURE may be found in "The life of HORACE BENEDICT DE SAUSSURE" by DAVID FRESHFIELD.

We are indebted to FRESHFIELD for the excellent portrait in Plate IV, and for figure 2 which indicates the material environment of NICOLAS THÉODORE during his early life.

NICOLAS THÉODORE, the eldest son of HORACE BENEDICT DE SAUSSURE, was born at Geneva, October 14, 1767. The boy was educated at home for a time because his father disapproved of the educational methods of the public schools. Later, however, he attended l'Académie de Genève, became very much interested in the natural sciences, and finally became his father's assistant and companion. After his father's journeys ceased, NICOLAS THÉODORE found time for his own botanical and chemical researches. The family fortunes were sufficient to permit considerable travelling so that NICOLAS

FIG. 2. The town house in Geneva where NICOLAS THÉODORE DE SAUSSURE spent much of his childhood.

THÉODORE, as well as his father, was in direct contact with the work of foreign scientists. The summer of 1793 he spent in England in company with distant relatives, but late in December of that year his mother informed him of the loss of the family fortune and advised him to seek a travelling tutorship, for his father was no longer able to provide him with the ease to which he had been accustomed. Such a tutorship was not to be found, however, for the English patrons were far more interested in the classics than in scientific studies, and NICOLAS THÉODORE returned to Geneva in the summer of 1794. He had not been home long when he and his brother were forced to flee to Rolle on account of the great disorder in Geneva resulting from the effects of the revolution in France. It was in Rolle in 1794 and 1795

that NICOLAS THÉODORE spent a great deal of time correcting proof of the last two volumes of his father's "Voyages." The following year he returned to Geneva, married RENÉE FABRI, and settled down once more to his scientific career. The treatise on carbonic acid in its relation to vegetation,
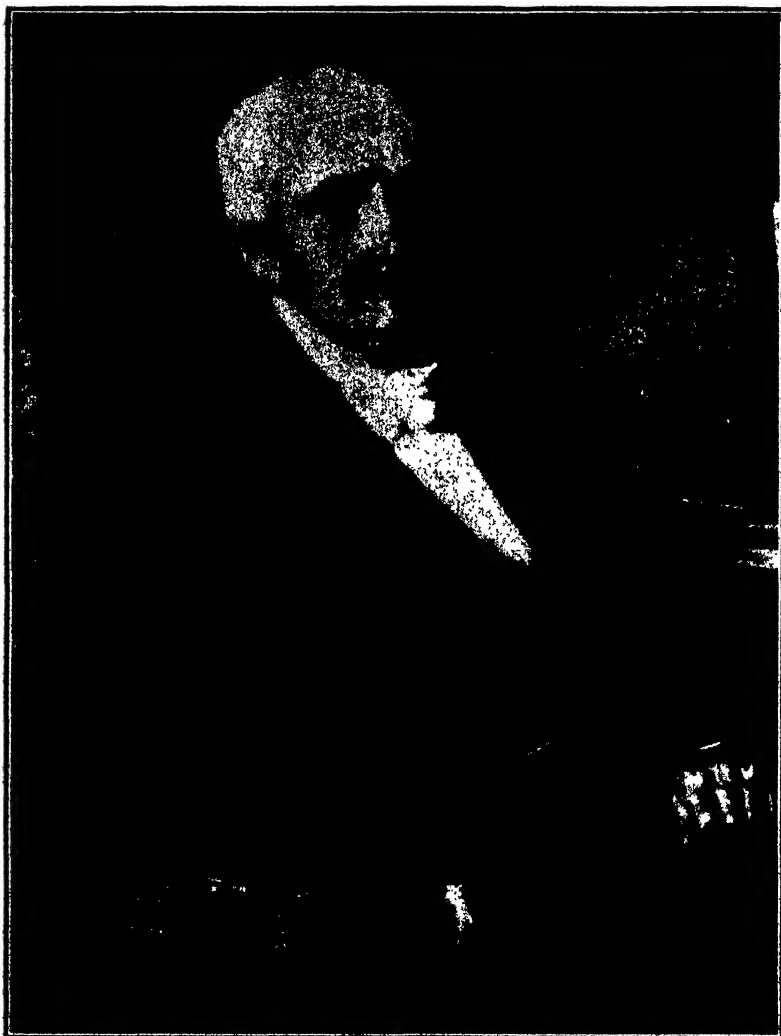
FIG. 3. NICOLAS THÉODORE DE SAUSSURE in later life as Professor of Mineralogy and Geology in the Academy of Geneva.

a prelude to his principal work "Recherches Chimiques," was written in 1797. After a short interval there was more travelling so that several years were spent in England and in France, and the wanderers did not return to

Geneva until 1802. At that time DE SAUSSURE received the appointment of Professor of Mineralogy and Geology in the Academy of Geneva. The position carried a stipend but was an honorary appointment with no regular duties; thus he was free to continue the work he had begun. His "Recherches Chimiques sur la Végétation," which was published in 1804, received great attention from the scientific world, but it was a long time before his ideas were completely understood and appreciated. The old humus theory had such a strong hold on the botanists of Europe that DE SAUSSURE's experiments were misconstrued and misunderstood until the reaction against the theory of a vital force set in twenty or thirty years after his publication.

DE SAUSSURE insisted that certain minerals found in the ash of plants were not accidental ingredients taken in with the soil water but were essential to the plant as foods in spite of the fact that they were often present in extremely small quantities. He recognized the fact that in the absence of any one of those essential elements development of the plant was impossible. He also was aware of the fact that the amount of an element was not the all-important consideration, but that a trace of one substance might be sufficient while larger quantities of other substances were needed.

Respiration was studied as well as carbon assimilation. Growth of the plants was impossible without respiration, and those parts in which physiological processes were most active required more oxygen than the parts in less active states.

NICOLAS THÉODORE DE SAUSSURE was a corresponding member of the French Institute and a foreign member of the British Royal Society. He was also a member of the Société de Physique et d'Histoire Naturelle of Geneva. Figure 3 is taken from a portrait painted during his later life. Most of his life was spent in Geneva and it was there that he died April 18, 1845. With INGEN-HOUSZ and SENEBIER, DE SAUSSURE is responsible for founding the modern theory of plant nutrition.—HELEN HART, *Division of Plant Pathology and Botany, University Farm, St. Paul, Minnesota.*

SOME ACCESSORIES FOR THE DISSECTING MICROSCOPE

(WITH ONE FIGURE)

The use of the Greenough type of binocular dissecting microscope may be greatly facilitated by the addition of the attachments described below.
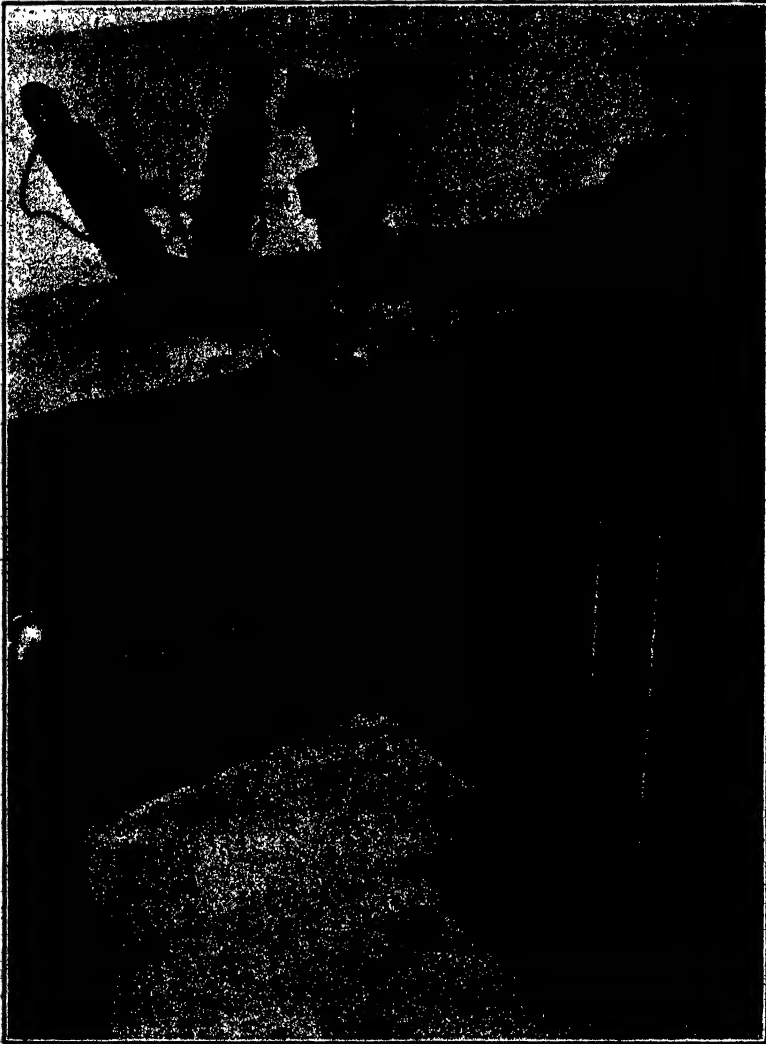
Fig. 1. Some accessories for the dissecting microscope.

In this arrangement the microscope, a Leitz UBM, is mounted on a table by means of a clamp stand *P*, placed in front of the operator.   By reversing the position of the stand and placing it on the opposite side of the micro-

scope from the operator even more working space can be obtained. The focusing of the instrument while dissecting is accomplished by the foot-operated mechanism shown. A shaft, fitted with ball bearings mounted in block A, has a pulley B on one end and is connected to the focusing wheel of the microscope by a rod with leather universal joints at C and D. The mounting for the wheel E is bolted to the table and the pulley F is belted to B. By having a tight belt and closely fitted universal joints lost motion is eliminated and the operator may focus the microscope as easily and as accurately as when the hand is used.

The proper illumination for dissection requires a beam of light low in heat. The lamps G and H, shown in the picture, are satisfactory. Osram 6 volt, 25 watt, Nitra globes supply the light, current coming from a transformer on the lighting circuit. An ordinary socket I holds the globe in position in a metal cylinder J, $2\frac{1}{2}$ inches in diameter. In front of the globe is a condenser composed of two lenses of two inch diameter and two inch focal length. The lenses are mounted in a sliding sleeve and are adjusted by moving the handle K attached to the sleeve and projecting through a spiral slot in the cylinder J. A cone-shaped nosepiece L to hold filters fits over the lower end of the cylinder. This lamp may be adapted for use with the compound microscope by using a ground glass filter.

For dissection work three lamps are used, two mounted either together or separately provide illumination from above, and one from below. This lower lamp which does not show in the picture is directed upward through a hole in the table top. Three small switches Q control the three lamps independently. A diaphragm set flush with the table top above the lower lamp regulates the size of field and aids in centering the object.

A mechanical stage M adapted to hold a petri dish provides for accurate movement of the object which is held in place by suitable spring wire clamps N and O. Dissection may be done in air or in water. A suitable shade around the oculars gives a brighter field and prevents eyestrain. This equipment has proved helpful in studying structural relationships in plants. It should also aid in microchemical testing and other work requiring manipulation of material while it is under observation.—A. S. CRAFTS, *Division of Plant Pathology, University of California, Berkeley.*

EFFECT OF SOMATIC INJURY UPON YIELD IN CORN[1]

Introduction

In a series of experiments on hens the author (5, 6) found the partial removal or mutilation of the ovary or merely bodily injury produced in some birds a stimulating effect in egg production. In approximately an equal number of birds in the same experiments the operation produced a retarding effect. When the operations were performed on six-weeks' old pullets,[2] similar stimulation and retardation were noted in the length of time until the first egg was laid.

In order to test for a similar reaction in plants a series of experiments was performed on corn. While the results do not warrant a conclusion directly related to the experiments on animals such as those described above and those of ZELENY (7), PEARL (4), HARTMAN (3), and others, yet some very interesting results from an economic point of view are indicated by the experiments. The disputes arising from some appraisals by insurance companies on hail or wind injured crops, the custom in some localities of allowing lambs to eat off the lower leaves of corn, seem to the author to be examples to which the results of this experiment may be related.

The author wishes to express his appreciation to Mr. SAM DUNKELBERGER, Halstead, Kansas, who harvested the corn in the fall and on whose farm the experiments were performed during the summer of 1929, also to his colleague, Dr. E. C. DRIVER of Smith College, for helpful crticisms during the writing of this paper.

Methods

The experimental plants were selected in a field of about four acres in size. The corn was between five and six feet tall and the silk was just beginning to appear when the experiments were begun. The plants of each experiment were tagged with different sun-fast cloth labels.

Each experiment, with its results is described below.

Results

EXPERIMENT 1.—A search was made to find 50 hills each containing 2 plants of equal height, thickness and quality. One stalk was to be the operated and the remaining one the control.[3] In the "operated" stalk 3 longitudinal incisions, about 3 inches long, were made through the stem at about 18 inches from the ground. The incisions were made from different sides of the stalk.

[2] Paper now in manuscript form.
[3] In case there was a slight advantage in one of the two stalks it was always given to the control. This procedure holds for the other experiments as well.

There were 45 hills recovered in November when the corn was harvested. The 45 operated stalks produced 48 ears which yielded 25 pounds of shelled corn. The 45 control stalks produced 45 ears of corn which weighed 25 pounds and 3 ounces when shelled. Obviously there is no significant difference in total corn yield in this experiment. During the summer it was noticed that there were several operated stalks considerably taller than their particular controls, yet this factor was not critically analyzed.

EXPERIMENT 2.—The material for this experiment was 25 hills each containing 2 similar plants. From one plant in each hill all the leaves were removed except the two nearest the tassel. These were called ''operated plants'' and their corn yield was compared with the control plants. The 23 operated stalks recovered carried 7 ears of corn which produced 1 pound 4 ounces of shelled corn as compared with 29 ears from 23 control stalks, which yielded 14 pounds and 5 ounces of shelled corn.

EXPERIMENT 3.—This experiment was similar in every way to experiment 2 except that every other blade was removed from the operated plant. There were 22 operated stalks recovered on which there were 19 ears which produced 5 pounds 10 ounces of shelled corn. From the 22 control stalks, 23 ears were obtained, the shelled corn of which weighed 12 pounds and 12 ounces.

EXPERIMENT 4.—Fifty individual plants were selected on which there were either 2 or 4 ears. In case there were 2, one of them was cut, directly down the middle, leaving only the back part of the husk to hold the ear somewhat intact. If there were 4 ears on the stalk every other one was cut. The uppermost ear on each plant was used alternately as a control and an operated ear. A few days after the operation the operated ears spread out, due to the lack of tension, causing the silk to appear through the incision. Soon the cob began to curl and the immature kernels became hardened by the weather. Many of these ears became so infected with molds that they were lost entirely.

There were 30 operated ears recovered which produced 2 pounds 12 ounces of shelled corn as compared with 39 control ears which yielded 20 pounds and 8 ounces of shelled corn.

The data described in these experiments are shown also on table I.

Discussion

Part of a letter from a farmer, who was acquainted with these results, appears below.

''In August we had a hail storm, when the ears of corn were partly made; the grain had already started on the cob. During the storm the blades were knocked off but the ears still remained. The insurance adjuster refused to grant my claim, saying that no damage was done to the actual

TABLE I

NUMBER OF EARS AND THE WEIGHT OF SHELLED CORN OBTAINED IN FOUR EXPERIMENTS ON
THE EFFECT OF INJURY ON CORN YIELD

EXPERIMENT	NO. OF EARS		SHELLED CORN		YIELD PER EAR	
	OPERATED	CONTROL	OPERATED	CONTROL	OPERATED	CONTROL
			lbs.	lbs.	lbs.	lbs.
1. Incisions through corn stalk	48	45	25.00	25.19	0.52	0.56
2. Every blade removed	7	29	1.25	14.31	0.18	0.49
3. Every other blade removed	19	23	5.63	12.75	0.30	0.55
4. Ear cut longitudinally	30	39	2.75	20.50	0.09	0.53

corn yield. The yield was actually lessened, and by your experiments I can prove my claim." It is obvious from table I that the more blades removed before the corn is ripe the greater is the damage. Some Iowa farmers allow lambs to eat off the lower leaves of corn. This experiment may have a bearing on this practice as well.

Although the yield was less in the operated plants, the results may still be related to the zoological experiments cited in the introduction. It was mentioned there that both a stimulation and retardation was noted. Perhaps in the work on corn only the retardation became evident. Some plants, *e.g.*, dandelions or willows, might experience some stimulation to a more luxuriant growth of leaves or of seed, when injured.

Conclusion

Corn plants which have been injured produce less corn than uninjured plants.—MORRIS STEGGERDA, *Smith College.*

LITERATURE CITED

1. DUNGAN, G. H. Effect of hail injury on the development of the corn plant. Jour. Amer. Soc. Agron. **20**: 51–54. 1928.
2. ——————. Relation of blade injury to the yielding ability of corn plants. Jour. Amer. Soc. Agron. **22**: 164–170. 1930.
3. HARTMAN, C. G. Observations on the functional compensatory hypertrophy of the opossum ovary. Amer. Jour. Anat. **35**: 1–24. 1925.

4. PEARL, RAYMOND, and SCHOPPE, W. F.   Studies on the physiology of reproduction in the domestic fowl.   XVIII. Further observations on the anatomical basis of fecundity.   Jour. Exp. Zoöl. **34**: 101–118.   1921.
5. STEGGERDA, M.   Effect of ovarian injury on egg laying in fowls.   Jour. Exp. Zoöl. **51**: 403–416.   1928.
6. ——————.   Effect of bodily injury on egg laying in fowls.   Poultry Sci. **9**: 79–91.   1930.
7. ZELENY, C.   A study of the rate of regeneration of the arms in the brittle-star, *Ophioglypha lacertosa*.   Biol. Bull. **6**: 12–18.   1903.

NOTES

The Annual Election of Officers.—During the last two months the annual election of officers of the American Society of Plant Physiologists has been in progress. The Secretary-Treasurer, Dr. H. R. Kraybill, of Purdue University, has just announced the results of the balloting, as follows:

President, Dr. H. R. KRAYBILL, Purdue University.

Vice-President, Dr. W. E. TOTTINGHAM, University of Wisconsin.

Secretary-Treasurer, Dr. WRIGHT A. GARDNER, Alabama Polytechnic Institute.

The year just closing has seen steady growth of the Society, and the new officers should find conditions pointing to a bright future. With the cooperation of the present membership it should be relatively easy to bring the membership to the 500 mark within the next year.

Committee on Physical Methods.—A committee has been organized to do for physical methods of research a service similar to that rendered by the Committee on Analytical Methods for the chemical methods of attack. It will be the purpose of the committee to summarize and possibly to recommend standard methods of procedure for biophysical research. The chairman of the committee is Dr. R. B. HARVEY, of Minnesota. The other members so far chosen are Dr. G. W. SCARTH, McGill, who will compile methods of protoplasmic research; Dr. L. O. REGEIMBAL, Minnesota, pH and conductivity measurements; Dr. D. R. HOAGLAND, California, nutrient solution methods. Dr. E. S. JOHNSTON, Maryland, osmotic pressure and general water relations; Dr. H. W. POPP, Pennsylvania State College, light. Other members may be added later, as the work of the committee develops. There is opportunity for a real service to plant physiology in this project, and the results of the work of collating methods will be awaited with much interest.

Life Membership Committee.—President KRAYBILL has asked the following members to serve on the CHARLES REID BARNES Life Membership Committee: Dr. S. F. TRELEASE, chairman; Dr. R. B. HARVEY, Dr. J. P. BENNETT, Dr. L. F. GRABER, and Dr. D. B. ANDERSON. The duty of selecting the sixth recipient of this honor will be the main function of the committee. The results of the selection in past years have been announced at the annual dinner of the physiologists, and a similar arrangement may be made for the Cleveland meeting in December, 1930.

The Purdue Section.—The Purdue Section of the American Society of Plant Physiologists has had a splendid year. With 31 members, 15 of

whom are members of the national organization, the average attendance has been about 25 at each biweekly meeting. It has been customary to have invited speakers at the first and last meetings of the year. Dr. E. J. KRAUS, of the University of Chicago was present at the first meeting giving a very helpful informal discussion of plant research. The speaker at the final meeting, was Dr. W. E. TOTTINGHAM of Wisconsin, who discussed the problems of temperature and metabolism, and led a discussion of the factors affecting winter hardiness after the lecture.

The programs for the regular meetings were as follows:

October 7, Crop nutrition studies in Europe, S. D. CONNER.
October 21, Physiology of the apple blotch fungus, E. J. KOHL.
November 4, Further studies on color and quality of tomatoes, J. H. MAC-GILLIVRAY.
November 18, Purification and properties of tomato mosaic virus, P. H. BREWER.
December 2, Recent respiration studies at the Cambridge Botany School laboratory, R. E. GIRTON.
January 13, Joint evening meeting with the Biological Society devoted to reports of the Des Moines meeting of the A. A. A. S.
February 3, The Neubauer method of soil analysis, S. F. THORNTON.
February 17, Fungus inhibition and staling, C. L. PORTER.
March 3, Some recent results of the corn stalk method for testing soil fertility, G. N. HOFFER.
March 17, Ozone treatment of fruit in storage, C. E. BAKER.

At the final meeting, the officers of the Section for the next year were installed. Dr. R. E. GIRTON is the chairman for 1930–31, and S. F. THORNTON, secretary. The Purdue Section has set a high standard for its activities, and deserves the fine success which has attended it.

The Minnesota Section.—The Minnesota Section also reports some excellent meetings on hardiness, light, etc. The April meeting occurred on April 15, and in addition to an address by Dr. CHARLES SHEARD, of the Mayo Clinic, on "Physiological effects of irradiation," several demonstrations of apparatus were made. Mr. HAROLD MITCHELL demonstrated the Illuminometer method of light measurement; Dr. L. O. REGEIMBAL demonstrated and discussed the Potentiometer Light Recorder, which uses General Electric Photoelectric Cells, and Dr. H. SHIRLEY demonstrated and discussed his new Radiometer. Dr. C. H. BAILEY is chairman of the Section, and R. B. HARVEY, Secretary.

Deaths of members.—We regret to announce the death of two of our members.

Patrick Beveridge Kennedy.—The death of PATRICK BEVERIDGE KEN-NEDY, Professor of Agronomy at the University of California, occurred on January 18, 1930. Born at Mt. Vernon, near Glasgow, Scotland, in 1874, he grew up with a love for agriculture, horticulture and botany, and began his education along these lines in Scotland and England before he came to America. He attended the Ontario Agricultural College, and finally received his first degree from the University of Toronto in 1894. After spending a short time at the Ontario Agricultural College as assistant chemist, he went to Cornell, where in 1899 he won his Ph.D. degree. He was in the Department of Agriculture for a couple years, and then went to Nevada, where he was connected with the work in botany, horticulture and forestry from 1900 to 1913. In 1914 he went to the University of California, where his lofty ideals of research and his rare love and understanding of the plant materials with which he worked attracted ever increasing numbers of graduate students, who found with him unequaled opportunities for study in the field of agrostology.

He spent much time on the taxonomic revision of the genus *Trifolium*, and was working on the section of the bladder clovers at the time of his death. Many monographs were written on grasses, saltbushes, forage plants, alpine plants, etc., and he was interested in the introduction of new grasses to the stock ranges of California. Harding grass, Kikuyu grass, Rhodes grass, and bulbous blue grass were among those brought in to improve the ranges. He was an authority on turfs, and his advice was widely sought in the management of greens on country club grounds. To his fellow members of the American Society of Plant Physiologists, he is best known for his work on the perplexing problem of control of the wild morning-glory by chemical sprays.

In spite of domestic cares, he was always cheerful, and his willingness to serve others endeared him to all who knew him. One of his greatest pleasures was the loyal friendship of his students and colleagues.

Grace Barkley.—The death of Dr. GRACE BARKLEY occurred on April 1, 1930. Death was believed to have resulted from a cerebral hemorrhage following a fall down a stairway. Although she lived for six days after the fall, she was never able to explain the circumstances.

She was completing her third year at DePauw University, where she had gone after receiving her Ph.D. at Chicago. Her main work was on the origin of spiral markings in the protoxylem of *Trichosanthes*. She had previously published on the origin and development of secondary wood in *Yucca*.

New Laboratory at the University of Chicago.—The work in plant pathology and plant physiology at the University of Chicago is moving into the new laboratory on Ingleside Avenue, which has been erected during the last six months in proximity to the new greenhouses which were built during the preceding summer. The laboratory is equipped for many different lines of work. Pathology has a general laboratory, research laboratory, refrigeration room, inoculation room which can be sprinkled down, and a preparation room. In addition it shares use of other rooms, as store room, apparatus room, etc. Plant physiology has a laboratory for elementary and general class work, a biochemical laboratory with adjacent balance room, nitrogen laboratory, biophysics laboratory, seed laboratory, microchemical laboratory, and radiation laboratory for ultra-violet, X-ray, and other short wave radiation research. There is also an oven room, and dark room for any work requiring dark conditions. There is also a well equipped photographic room and dark room, providing facilities for every research requirement along photographic lines.

Temperature control rooms are located in the basement, with a possibility of −40° C. at the lowest. A grinding room will be developed in the basement, and soil centrifuge for work in soil physics.

The greenhouses are also provided with temperature and humidity control chambers, and soil temperature control apparatus. These new laboratories offer unique opportunities for investigation in plant physiology and plant pathology. As a plant of this sort is appreciated more when seen in operation, investigators passing through Chicago are invited to see the new plant. It will be ready for complete occupation by the first of October. Classes are using the instructional laboratories this summer.

Statistical Methods for Research Workers.—The third edition of R. A. Fisher's monograph on "Statistical Methods for Research Workers" has come out after the second edition was only 2 years old. Naturally the treatment of statistics does not change rapidly enough to warrant frequent editions, and the multiplication of editions without sufficient reason ought to be discouraged. Two new subsections, comprising about 10 pages, are added to section 57. These new paragraphs deal with fragmentary data, and the amount of information, design, and precision; they illustrate some wider applications of the method of maximum likelihood, and the quantitative evaluation of information. The author has also added a section of citations of his own papers, whether pertinent or not. These changes indicate not sufficient reason for a new edition. The price of the book is 15 shillings, and orders should be sent to Oliver and Boyd, London.

General Physiology.—A textbook of general physiology suitable for beginners has been prepared by Dr. G. W. Scarth, and Professor F. E.

LLOYD, of McGill University. The text (194 pages) is accompanied by laboratory exercises, which occupy 60 pages in the back of the volume. There are 14 chapters, with the following headings: Life as a mechanism; organization of protoplasm; surface tension in physical systems; surface tension in cells; adsorption in physical systems; adsorption in cells; diffusion and osmosis in physical systems; diffusion and osmosis in cells; ions and their determination in physical systems; ions, particularly H-ions, and their determinations in cells; electric potential and electric currents in physical (non-metallic) systems; electric potential and electric currents in cells; colloids in physical systems; colloids in the cell. The laboratory outlines are arranged in 13 groups, the first eleven of which cover the ground of chapters 2–14. The last two outlines deal with ultramicroscopy, and the general properties of enzymes. The treatment is simple, and should furnish a stimulating introduction to the field it covers. Naturally there are a number of topics one might expect to find in a general physiology which have been omitted. Nevertheless, the text will offer many good suggestions for class experiments along lines that never have been emphasized sufficiently in teaching plant physiology and general physiology. The price quoted for the text is $2.75, and the publishers are John Wiley and Sons, New York.

New Tables for Reducing Sugars.—A little volume of Recalculated Tables for the Determination of Reducing Sugars by Bertrand's Method has been published by the author, Dr. Z. I. KERTESZ, of the Department of Agricultural Chemistry, the Agricultural Experiment Station, Geneva, N. Y. It gives in both English and German, complete and exact directions for the Bertrand method for reducing sugars. It should be very useful for the non-chemist as well as the chemist. In the original tables the even increments were in terms of sugar. Here they are for Cu, from 1.0 to 190.0 mg., by 0.2 mg. increments, making the tables far simpler to use. The sugars include glucose, invert sugar, maltose, lactose, mannose, galactose, sorbose, arabinose and xylose. This method is commonly in use in Europe and is becoming more and more favored in this country. The volume contains 34 pages, and can be purchased for 75 cents, from the author.

Exploring for Plants.—Although not specially useful to plant physiologists the editor cannot refrain from mentioning two books which have recently come to his notice. One of these, ''Exploring for Plants,'' is by DAVID FAIRCHILD, who has for many years explored the earth for useful plants. This interesting book is a record of the Allison Vincent Armour expeditions for the Department of Agriculture during 1925, 1926, and 1927.

The life of a botanist is full of romance, and this story by FAIRCHILD is one of the romances that everyone will want to enjoy. It is told in 43 captivating chapters, and beautifully illustrated. For $5.00 one can enjoy one of the most interesting botanical stories of the twentieth century. The publishers, Macmillan Co., New York, have done an excellent piece of work in the make up.

Rock Gardens and Alpine Plants.—The other work referred to above, is HENRY CORREVON's "Rock Garden and Alpine Plants," also from Macmillan. HENRI CORREVON is the greatest living student of alpine vegetation. His first book on the subject was published in 1884, and he has had a constant interest in the subject for more than 50 years. The book is a storehouse of information for anyone interested in rockeries, and the problems of acclimatisation and cultivation of mountain plants in lowland gardens. There are 15 illustrations, eight of which are colored plates, one thinks not any too true to the natural colors. If for no other reason, one should read it for the glimpse it gives into the romantic interest in plants in the life of its distinguished author, HENRI CORREVON. The quoted price of this work is $6.00, and orders should be sent to the publishers.

Physiology and Biochemistry of Bacteria.—Received too late for extended notice in this number of PLANT PHYSIOLOGY, we call attention to the publication of the last two volumes of this valuable work on the physiology of the bacteria by R. E. BUCHANAN and E. I. FULMER. The first volume was noticed in the April number of this journal, 1929. The set as a whole represents an enormous amount of work, and the authors have made a permanently valuable contribution to our knowledge of bacterial life. A more extended notice will be given in the October number. Those who desire the complete work should address Williams and Wilkins, Baltimore, Maryland. The last two volumes are $7.50 each.

PLANT PHYSIOLOGY

OCTOBER, 1930

THE FEEDING POWER OF PLANTS[*]

WALTER THOMAS

A. Introductory

1. VARIABLE COMPOSITION OF IDENTICAL SPECIES

The publication of WOLFF's monumental work on "Aschen-analysen" in 1871 (**210**), although compiled principally for the purpose of supplying information on the composition of plants used in feeding farm animals, nevertheless, served to crystallize the large amount of experimental data of English, French and German investigators that had accumulated even in those early days of agricultural science. WOLFF's data showed very clearly that plants even of the *same species* differed considerably both in the *absolute* and *percentage amounts* of the various elements absorbed, depending upon the composition and nature of the substrate (soil or nutrient solution). Indeed, WOLFF himself (**209**) was the first to give definite proof that the composition of the ash of any plant could be changed by varying the proportion of salts in the nutrient culture medium.

2. SELECTIVE POWER OF DIFFERENT SPECIES

Numerous investigations, those of NEWTON (**129**) being the most recent, have since shown, moreover, that *different species* growing in media of identical composition possess selective powers with respect to any specific ion or ions. This marked difference in the assimilatory powers of different plants is very clearly exhibited in their different responses to such "insoluble" mineral fertilizers like rock phosphate, basalt, gneiss, etc.

3. ARE DIFFERENCES IN RESPONSE A SPECIES CHARACTERISTIC

But as the environmental conditions even in the water and sand culture experiments cited by WOLFF were never identical, the question as to the

[*] Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station as technical paper no. 502.

nature of the factors operative was still left open. Nor was it decided whether the differences in responses exhibited were characteristic of the species or the result of environmental or other unknown factors. And, although NEWTON (126) believes that with the exception of phosphorus (129) there is no characteristic difference in the *type of absorption* of certain species of plants (barley and peas, for example) in nutrient culture solutions, *i.e.*, that there is no difference in percentage content with respect to any element in the plants examined that holds consistently in favor of one or the other throughout all stages of growth, it will be apparent as the subject is developed that the evidence is overwhelming that different plant species growing in media of identical composition not only exhibit differences in composition that can only be interpreted to indicate marked differences in the type of absorption (5) but that they exhibit also differences in what has been frequently designated in the literature by the expression feeding power or ability to utilize insoluble soil constituents to different degrees, which are characteristic of the species. The following data from KÖNIG's experiments (82)—which are among the earliest—are typical as exemplifying this point:

TABLE I

PER CENT. OF K$_2$O IN OATS AND PEAS GROWN UNDER SIMILAR CONDITIONS

		SANDY	LOAM	LOAM	CALCAREOUS	CLAY
Yields (first year)	Oats	0.396	0.506	0.420	0.489	0.401
	Peas	0.554	0.688	0.554	0.666	0.702

4. THE TERM "FEEDING POWER" DEFINED

The term "absorbing power" or "feeding power" as it is known in technical agronomy may connote several ideas (67). Many have objected to the use of the term feeding power. SHULL (172) suggests that some plant physiologists prefer not to use the term on the basis that it has too much psychological implication and others (67) because of the many different interpretations given to it. But, as CROCKER (27) points out, although the expression has undesirable implications, it is better to continue its use than to invent some new phrase that might, perhaps, be freer from these implications, because other terms suggested, like "absorbing power of crop plants" or even "specific absorbing power," do not imply quite enough. The term "feeding power" implies both power to absorb the soil nutrients and also the power of making use of many of the salts absorbed. This expression will be used in the present paper as a concise means of indicating

the difference exhibited by different types of plants in the *absorption* and *utilization* of difficultly available minerals, either native to the soil or added as soil amendments, as determined by the differences in the amount of nutrient elements absorbed from the minerals in question, the utilization of which is generally exhibited in different growth responses.

5. THEORIES ADVANCED TO ACCOUNT FOR DIFFERENT RESPONSES

From the numerous experiments conducted to determine the cause of the characteristic differences in absorbing power by different species, a number of explanations have been advanced. The differences have been attributed to (1) the differences in the chemical nature (and possibly amount also) of other products of root exudations (acids, carbohydrates, etc.); (2) the differences in (a) the absolute amounts of carbon dioxide evolved, or (b) the amounts evolved per gram of dry weight of the roots (respiration energy); (3) the operation of the law of mass action between the carbon dioxide (carbonic acid) from the roots and the "insoluble material" in the soil; (4) unlike potential differences between the soil and plant; (5) differences in the permeability of the root membranes producing "selectivity" in absorption of roots; (6) the existence of a DONNAN membrane equilibrium, and to a combination of one or more of the above factors (67).

We shall discuss the evidence for the operation for these various factors separately.

B. The chemical nature of the root exudations

1. THE CARBON DIOXIDE FACTOR

The problem of determining the nature of root secretion is of importance not only from the standpoint of scientific inquiry, involving the fundamental question of the mechanism of absorption of nutrients and the reaction of protoplasm, but is of importance also from the practical aspect with respect to the economic utilization of minerals occurring naturally in the soil, and applied also as soil amendments.

a. CONSIDERATIONS OF SOME OF THE EXPERIMENTAL DIFFICULTIES INVOLVED.—It must be granted that even if proof were forthcoming that carbon dioxide is the only factor involved, as indeed HALL (55) points out, the problem of isolating the factors that contribute to the sum total of the soil carbon dioxide is not a simple one. In the first place, the carbon dioxide is not all in the gaseous phase, a large part (163) (and, indeed, under some conditions nearly all) is present in solution (93); and in the second place, the soil respiration is the total of all the soil processes that give rise to the production of CO_2, viz., oxidation (83, 169, 213), the metabolic processes of the microorganisms (110, 181, 182, 183, 213), and the

respiration of roots and of the bacteria that inhabit them (58, 63, 110, 178, 181, 208). It follows that the concentration of carbon dioxide, as measured in most experiments conducted with soils, is a function of the absolute CO_2 produced by the soil particles together with the roots, and also of the diffusion velocity (102, 103).

Since the distribution of the CO_2 between the gaseous and liquid phases will depend upon a number of variables (water and calcium principally), the net effect of any one factor at any time is necessarily difficult to determine. RUSSELL (162) believes that the CO_2 evolved from plant roots cannot be distinguished from that given off by microorganisms and this is also the conclusion drawn by STARKEY (177, 178); but the work of STOKLASA (182), HEADDEN (60), TURPIN (198), BARAKOV (3) and NELLER (124) indicates that approximate relative differentiation is possible.

b. THE VIEWS OF THE SEVERAL SCHOOLS.—Plant physiologists and soil scientists have been and still are divided into several schools. One group (10, 155, 162) maintains that plants do not dissolve *significant* quantities of minerals that would otherwise remain undissolved; another school emphasizes the important rôle that the root exudations exercise in this connection. The latter school is divided into two groups: One (26, 38, 39, 49, 77, 89, 96, 141, 145) subscribing to the view that "insoluble" substances are rendered soluble by the exudation from the roots of plants of substances (*e.g.*, organic acids, acid salts, etc.) other than carbon dioxide; and the other school (1, 52, 55, 60, 117, 121, 182) that only CO_2 is exuded from the roots and that the influence of plants on the solubility of soil minerals is to be attributed solely to this factor. The rôle played by microorganisms in the soil and especially the action of microorganisms of the rhizosphere, isolated by STOKLASA (181) is recognized by all schools.

c. CHARACTERIZATION OF OBJECT OF EXPERIMENTS IN THIS FIELD.—Consequently, in the examination of the available evidence for the purpose of evaluating the contribution of the carbon dioxide evolved from plant roots as a factor in dissolving the "insoluble" soil materials, we must distinguish between (1) those experiments (3, 31, 59, 60, 84, 85, 86, 92, 102, 103, 163, 182, 198, 211, 212) that have for their purpose the determination of the relative contribution of carbon dioxide by root and microorganisms of the soil, and (2) those (28, 38, 49, 77, 89, 117, 121, 145) which, although accepting the important rôle of the carbon dioxide evolved from the roots, were carried out for the purpose of determining if acids and other substances than CO_2 are exuded by roots, and (3) those (37, 139, 140, 181, 191) that are concerned with the effect of CO_2 from whatever source derived.

d. OBSERVATIONS OF THE EARLIER INVESTIGATORS.—The older "corrosion" experiments of SACHS (165), KNOP (80), LIEBIG (97), KNY (81),

CZAPEK (**28, 29**) and MOLISCH (**122**) and observations of the natural etchings frequently found upon calcareous stones lying in the - soil were regarded as furnishing *prima facie* evidence that the roots of plants participate in dissolving "insoluble" nutrients of the soil.   And more recently FRED and HAAS (**44**) found that this action of roots on marble is increased when a sterilized soil was inoculated with pure cultures of various bacteria.

The growth fusion ideas (**26, 176**) and etching experiments of SACHS (**165**) and of CZAPEK (**29**) are of interest here.   The former observed that only the smooth surfaces of the minerals that were soluble in carbonic acid showed "corrosion figures"; silicates did not.   CZAPEK (**29**) sought further information on the problem by substituting plaster of Paris slabs which were incorporated with various carbonates and phosphates.   He argued (**28, 29**) that since—in addition to the carbonates—the phosphates of calcium, magnesium and iron, but not of aluminum phosphate, were corroded by roots, the acids exuded from the roots must be limited to carbonic, acetic, propionic and butyric.   Finally, all but carbonic acid were eliminated by means of qualitative results with Congo red.   CZAPEK'S deduction from these experiments, however, is not valid, for the evidence from the experiments of CAMERON and HURST (**20**), PRIANISHNIKOV (**151, 153, 155**) and MARIAS (**107**) is conclusive that aluminum phosphate is available to plants through hydrolytic processes.   The use, therefore, of $AlPO_4$ is not justifiable in experiments to decide the nature of root exudations.

Moreover, all these "corrosion" experiments may be criticized on the basis that no mineral phosphates exist that are unavailable to plants when all factors that hinder the dissolving power of the roots are removed.   The experiments of BUTKEWITSCH (**16**), which will be discussed later in this paper, exemplify this very clearly.

e.   THE USE OF SEEDLINGS IN DETERMINING THE NATURE OF ROOT EXUDATIONS.—The approach to the problem by the use of seedling plants, moreover, is not sound (**1, 28, 29, 52**).   The mineral requirements of such young plants are too small to justify the suitability of such experiments to answer the question of root exudations, by whatever method and however carefully carried out.   The conclusions from such experiments must be of doubtful value.   For this reason we shall consider only those experiments in which mature plants were used.

f.   THE PROBLEM OF INTERPRETATION.—The results of field plot experiments (**59, 60, 92, 102, 103, 163, 211**) are contradictory, but pot and lysimeter experiments (**3, 92, 128, 182, 198**), with certain exceptions (**86**) where the technique adopted may be open to criticism (**55**), agree in attributing to the roots a considerable and, indeed, preponderant contribution to the carbon dioxide of the soil.

(1) THE WORK OF THE ROTHAMSTED GROUP.—Notwithstanding the fact that on the Broadbalk field and Hoos plots RUSSELL and APPLEYARD (163, 164) found, in agreement with other investigators (3, 9, 59, 60, 92, 198), that at certain seasons of the year, especially at the time of active growth and ripening, cropped soils contain a higher content of CO_2 than uncropped soils, their interpretation of the results differ. RUSSELL and APPLEYARD (163) attribute the conflicting results of the older workers (40, 41, 123, 211), even when comparison was made between cropped and fallow portions of the same plot, to failure to recognize the marked influence of differences in the physical and chemical structure on the carbon dioxide content of the soils in question. The Rothamsted investigators conclude that in their own experiments the difference in the physical structure and composition of the cropped (or grass) and uncropped (fallow land) soils makes comparison difficult. These authorities observed that the smaller the soil differences become, the smaller is the effect of the crop on the production of CO_2 and they postulate that, if it were possible to obtain absolute identity of conditions, the effect of the crop would vanish. But that the factor assigned by these authorities for the observed differences cannot be the only one is shown by the work of HEADDEN (60), who found a marked depression of soil carbon dioxide after each cutting of alfalfa. There is, moreover, such a marked difference in the amount of CO_2 evolved from an uncropped and a cropped plot in some of the more recent field experiments (59) that it is difficult to conceive how such marked divergence could be due to physical differences in the soil of the plots. For example, HASSE and KIRCHMEYER'S (59) results indicate that four-fifths of the total CO_2 was due to root respiration.

(2) ARE LYSIMETER RESULTS APPLICABLE TO FIELD CONDITIONS?—Moreover, if RUSSELL and APPLEYARD'S (163) conclusions are generally applicable, the question may be raised whether pot and lysimeter experiments of the type conducted by BARAKOV (3), TURPIN (198) and others (9, 37) are applicable to field conditions. That the deductions drawn from some of these experiments may not be applicable to field conditions, on account of the conditions being too artificial, is possible. This criticism would apply to the conclusions of KOSSOWITSCH (84, 85, 86) in which CO_2 was determined in percolates of the nutrient solution in quartz sand cultures, but for the fact that DUSTMAN (37) has shown that, although the absolute respiration of plants grown in quartz and soil cultures is greater in the former, the relative differences between different plants are the same in both.

In TURPIN'S lysimeter experiments (198) the difference between the amount of carbon dioxide in the cropped soil and that in the uncropped

soil at the *period of most active crop growth,* divided by the amount of water transpired by the crop, gave a constant which varied with the season. Moreover, as there was no indication that the increased CO_2 in the cropped soil arose from the decomposition of root particles, TURPIN logically concludes from these experiments that the plant often produces at the period of its most active growth many times as much CO_2 as is produced by soil organisms.

The results of BARAKOV's lysimeter experiments (3), in which weekly determinations of CO_2 were made on different types of unsterilized soils, also indicate that the principal source of CO_2 is from the respiration of roots. The greatest absolute quantity of CO_2 was associated with the greatest development of the plant. He notes that each plant experimented with had its own specific respiration curve.

STARKEY (177, 178), however, frequently found little effect on the CO_2 content of the soil or abundance of organisms, especially in the early stages of growth. He also noted the occurrence of larger numbers of micro-organisms about plant roots than at a distance from them. On the basis of these findings STARKEY questions the conclusions of TURPIN (198) and BARAKOV (3). There is no reason to doubt that at certain periods a part of the carbon dioxide evolved in the respiration of roots is contributed by the bacteria that inhabit the root surface, *i.e.,* the rhizosphere (58, 110, 180); but the weight of evidence (3, 60, 198) indicates that the total contribution to the soil CO_2 by such means is relatively insignificant compared with the amount evolved during the growing season by the respiratory activity of the roots. It is also apparent on physico-chemical grounds that determinations of carbon dioxide on samples of soil taken under such conditions as those described by STARKEY (177) afford no basis for drawing conclusions concerning the quantitative relations relative to the carbon dioxide of the soil atmosphere *in situ.* This same disadvantage applies also to METZGER's (111, 112) attempt to measure the concentration of bicarbonates in soil samples immediately around the roots with those taken away from the roots, for these methods obviously destroy the CO_2 equilibrium conditions actually existing in the soil. A review of methods used for the determination of carbon dioxide of the soil atmosphere will be found in a paper by POTTER and SNYDER (150)

HUTCHINSON (72) concludes that there exists a parallelism between bacterial numbers and CO_2 content of the soil. This report (72) is frequently cited in the literature; but it is sketchy and lacking in details. If the exact parallelism between soil CO_2 and bacterial numbers occurs throughout the growing season, this fact would tend to show that the root contribution would be small; but when such correlations are attempted

(**72, 163**) there are well defined periods when the bacterial numbers fall as the CO_2 increases. If, then, as WOLLNY and others (**144, 198, 213**) suggest, relatively large increases of CO_2 depress bacterial activity, no correlation would be expected, which may, as PLUMMER (**149**) suggests, be due to the limitation of the oxygen supply. It should be noted, however, that STOKLAS (**180**) finds that root respiration and the number of bacteria in the rhizosphere paralleled one another.

We must, therefore, conclude that either RUSSELL and APPLEYARD'S (**163**) results were due to conditions not readily duplicated and that the conclusions drawn by them are not of general application, or that deduc-. tions from experiments of this type carried out in pots and lysimeters are not applicable to conditions in the field. The assimilation and utilization of nutrients in pot experiments is considerably greater than in field experiments. In the former the roots penetrate the soil more intensively than in the open field. As a result of this better utilization of nutrients, the yields in pot experiments are frequently six to ten times higher than those in the field. Recent suggestions (**32**) to eliminate these differences include mixing the soil with equal parts *by volume* of quartz sand. Finally, it is to be noted that there is no evidence that the discrepancies in the experiments discussed above can be attributed to the relative predominance of root respiratory activities as a result of the relatively low rate of oxidation of organic matter or *vice versa*.

g. INTENSITY OF CO_2 PRODUCTION BY ROOTS OF DIFFERENT SPECIES.—Since the atomic groupings and reciprocal linkages of the roots of different plants are not similar, it could be postulated that the respiration activity of the root systems of plants would be different. The complexity of the problem is appreciated when specific data for the respiratory energy of the roots of different plants are considered. The causes of the wide variations reported by different investigators for identical types of plants must be sought in the different conditions of experimental technique, especially with respect to the difficulty in making a comparison of the influence of the various factors that influence respiration, such as concentration of oxygen, temperature, concentration of carbon dioxide, and nature and concentration of nutrients.

Thus, the average amount of CO_2 evolved in 24 hours from barley grown to maturity was found by STOKLASA and ERNEST (**182, 183**) to be 70 mg. per gm. dry weight of roots, whereas NEWTON (**127**), in plants 35 days old, found only 3.5 mg. of CO_2 per gm. dry weight of roots evolved in 26 hours. Both of the above investigators used sand as the substrate. STOKLASA used sterilized sand and KNOP's nutrient solution. NEWTON used HOAGLAND's culture solution. NEWTON has thought that possibly his method

of simple diffusion may not have measured all the CO_2 evolved (130). But the agreement between DUSTMAN'S (37) results and NEWTON'S shows that this is not the reason and, theoretically, no great difference would be expected to result between systems depending on diffusion and air currents, respectively, since the measurements must necessarily have been made when each system was in a state of equilibrium between output and removal of CO_2 (104); nor is there any evidence that these large differences in STOKLASA'S and NEWTON'S values can be attributed to any great difference in the age of the plants used. Values obtained by the writer (186) for sweet clover in sand cultures by a similar method to that used by PFEIFFER and BLANCK (145) ranged from 8–9 mg. per gram. of dry weight of roots evolved in 24 hours, compared with values of 4.5 mg. obtained by NEWTON. DUSTMAN'S (37) results are in relative agreement with NEWTON'S. That STOKLASA and ERNEST'S (182) values are far higher than is usual under field conditions will be evident from a consideration of the following facts: STOKLASA concludes that the microorganisms in an acre of soil to a depth of 1.5 feet may produce between 65 and 70 pounds of CO_2 a day for 200 days in the year, and that during the growing period the roots of oats or wheat would give off nearly as much to an acre. This would mean a loss from decomposition of organic matter of 13,500 pounds of CO_2 per acre per year, or $0.471 \times 13,500 = 6,358$ pounds of organic matter. This value is abnormally high. As NEWTON (130) points out, a loss of one-half to one ton of organic matter per acre per year is closer to the average loss from normal soils.

It must be admitted (37) that calculation of the carbon dioxide production per gram of dry weight of material is not a very refined method of measurement, owing to the variation in the percentage amount of protoplasm in the various plants and parts of plants, the respiratory gas exchange of which depends entirely upon the activity of the living substance. Some tissues, such as wood and cork, consisting for the most part of dead cells, do not respire at all. But, owing to the impracticability of the alternative methods proposed (87), the simple method of calculation on the basis of total weight must be used.

Studied from a *relative standpoint*, however, STOKLASA and ERNEST'S results are decidedly valuable. Thus, the specific respiration energies (amount of CO_2 evolved per gram of dry weight) of roots found by them were barley 68, wheat 90, oats 127, rye 118, buckwheat 227. In sterilized sand culture experiments they found that the above values were in the same relative order as the utilization of P_2O_5 and K_2O from basalt and gneiss. As already pointed out, BARAKOV (3), too, in experiments with lupin, oats, barley and rye, found that the capacity to absorb Ca from rock

phosphate paralleled their values for the specific respiration energy. STOKLASA and ERNEST, whose results are in substantial accord with those of BARAKOV's (3), point out that the values found by them for the respiration energies do not, however, afford any indication of the relative yields under conditions where readily assimilable nutrients are supplied. Thus, in KNOP's nutrient solution the yield of barley was found to be far greater than that of wheat, rye or oats. This might be attributed to the compensating factor of extensive root development in the case of barley, as HESSE's measurements (62) of the root hairs of barley, oats, rye and wheat indicate and which are in harmony with the measurements of NOBBE (131).

TABLE II

SIZE OF ROOT HAIRS ON CEREAL CROP PLANTS

	Length	Diameter
	mm.	*mm.*
Barley	1.40	0.0102
Oats	0.98	0.0090
Rye	0.86	0.0070
Wheat	0.62	0.0068

If STOKLASA and ERNEST's results can be corroborated we could postulate: (1) That when the rate of supply of N, P and K are sufficiently rapid, as in the case of access to an ample supply of easily soluble nutrients, the yield will be proportional to the extent of root system. (2) But if one of these elements, such as phosphorus, is present only in a difficultly soluble form, the yield will be proportional to the respiration energies.

If these conclusions of STOKLASA and ERNEST (182) and of the other investigators cited (p. 446) can be proved to be of general applicability to our economic plants, we should be forced to accept the views of the school that regards the rôle played by carbon dioxide as the greatest factor determining their feeding power. The need, therefore, for more exact information on the respiration coefficients of the various types of economic plants is apparent.

2. To WHAT EXTENT DOES CO_2 FUNCTION AS A SOLVENT

a. THE IMBIBITIONAL WATER OF THE ROOT HAIRS IS NOT A SATURATED SOLUTION OF CARBON DIOXIDE.—ABERSON (1), on the basis of titration and hydrogen-ion concentration values obtained on *seedling roots*, concluded that the hydrogen-ion concentration of the water of imbibition of the mucilaginous "membranes" of the root hairs in close contact with the soil particles contains a saturated solution of carbon dioxide. Experimen-

tal evidence is cited to show that a saturated solution of CO_2 is able to bring into solution the insoluble soil minerals, especially phosphates.

But ABERSON's view is not valid for the following reasons: (1) A saturated solution of carbon dioxide has a hydrogen-ion concentration of 1.2×10^{-4}; this exceeds the hydrogen-ion concentration values of any of the root exudations found by ABERSON.   (2) With any given content of carbon dioxide in the soil atmosphere it is possible to calculate the content of CO_2 and HCO_3 ions in the water of imbibition in contact with it.

Since $HA = H^+ + A^-$ \hfill (1)

$$\frac{[H^+]\ [A^-]}{[HA]} = K_a \hfill (2)$$

$$\therefore [H^+]^2 = K_a \cdot [HA] \text{ or } [H^+] = \sqrt{K_a \cdot [HA]} \hfill (3)$$

and $[H^+] = \sqrt{K_a \cdot (C - [H^+])}$ \hfill (4)

where C is the concentration of the original acid.

Since $[H^+]$ is small, we have

$$[H^+] = \sqrt{K_a \cdot C} \hfill (5)$$

or $pH = -1/2 \log. K_a + 1/2 \log C$ \hfill (6)

For polybasic acids the first ionization constant may be taken, since the second ionization constant is small compared with the first.

Since the HENRY coefficient at N.T.P. is approximately $1:1$ (199), the percentage by volume of carbonic acid of the soil solution and the percentage in the soil air are approximately identical.   Hence, even if the soil air contained as high as 10 per cent. CO_2, the soil solution (if it consisted of pure water) would also contain 10 per cent. by volume of CO_2, since the HENRY coefficient is $1:1$. The pH of such a solution calculated from the above equation would be 4.10. Since, however, it is a solution of salts, and especially of bicarbonates, the dissociation of the carbon dioxide would be depressed even below the above value. Moreover, ABERSON has neglected to consider the influence of the salts present in the cell sap of the roots.

b. HOAGLAND's VIEW.—The data of HOAGLAND et al. (68, 70, 170) would seem to afford evidence that CO_2 may not play an important rôle in all soils. These investigators found no measurable change in the reaction of soils of such markedly acid character of which the hydrogen-ion concentration is of the order of magnitude of the dissociation constant (3.0×10^{-7}) of carbonic acid (200), when such soils are partially saturated with carbon dioxide. In soils having a lower hydrogen-ion concentration (higher pH), however, the change in reaction due to partial saturation with CO_2 was

quite significant. The decomposition of stall or green manures in *acid* soils of hydrogen-ion concentration of the above order of magnitude would, therefore, not be expected to increase the hydrogen-ion concentration of the soil to such an appreciable extent as to have any effect on the solution of "insoluble" materials. Inasmuch as there is an overwhelming amount of evidence from field and laboratory experiments that decomposing matter is a potent source of rendering the "insoluble" minerals of the soil available, the rate of decomposition varying with the age and nature of the material (108) and also nature of fertilization (206), we should—on the basis of HOAGLAND and SHARP's data—be forced to attribute the major favorable effect in the majority of soils not to carbonic acid but to the intermediate products of decomposition by the soil microorganisms, such as butyric, acetic and lactic acids—the dissociation constants of which are of the order 1.8×10^{-4} to 1.7×10^{-5}—and possibly also to replacement effects in the exchange complex.

HOAGLAND (68) sums up the situation as follows: "It appears to me that some soils are so acid that CO_2 is of slight effect, and that in other soils the nature of the soil minerals is such that CO_2 may have very little influence in promoting the solution of PO_4 or K; but, nevertheless, that CO_2 is of great general significance because of the existence of a great number of soils—not inconceivably the majority of soils—where CO_2 does appreciably alter the culture medium of the plant. In all this, we presumably must constantly keep in mind the difficulty of knowing just what the chemical system, and influence of CO_2, is at root-soil particle interphases." Yet it is evident from more recent field experiments (166) that there are exceptions even to this generalization.

The influence of nitrification processes must, however, not be underestimated (187). In soils rich in calcium, nitrification processes are generally accompanied by the destruction of bicarbonates, *i.e.*, with a decrease in the buffer value and increased utilization (p. 466). In practice, then, the aim should be made to increase the decomposition of organic matter without greatly increasing the calcium concentration of the soil solution. One way of doing this would be to apply lime in small amounts.

c. EXPERIMENTS ON THE INFLUENCE OF ARTIFICIAL ADDITIONS OF CARBON DIOXIDE TO THE SOIL.—It might be expected that the results of experiments in which definite quantities of carbon dioxide are introduced into the soil in which plants are growing would give results from which definite conclusions could be drawn; but this is far from being the case. LUNDEGÅRDH (103) has shown the beneficial effects of increasing the carbon dioxide concentration of the atmosphere surrounding the external assimilatory organs, and BREAZEALE and BURGESS (11) noted increased absorption of

PO_4 by seedlings from "floats" when saturated with CO_2. The results obtained by MITSCHERLICH (118), PFEIFFER and BLANCK (145), and also by PARKER (140) are, however, decidedly negative with respect to its influence on the absorption of difficultly soluble materials; even distinct injury has resulted in some experiments (118, 145). These negative results, however, afford no proof that the carbon dioxide produced under field conditions does not play an important rôle in the assimilation of mineral nutrients. As TRUOG (50) suggests, the equilibrium conditions governing carbon dioxide evolution in the soil at the interface of the plasma membrane of the root hairs may not as yet have been duplicated in the laboratory.

It is unfortunate that all investigators have used either quartz sand or soils low in colloidal material in which the factor of ion replacement does not play the important rôle that it does in soils high in colloidal material. In view of the fact, moreover, that carbon dioxide is well known to have toxic effects when the CO_2/O_2 ratio is relatively high (21, 24, 56, 118, 145, 167), this possibility must also be considered in the experiments hitherto conducted on the effect of CO_2 additions. PFEIFFER and BLANCK (145) observed distinct injuries, especially to oats, in their first series of wheat, lupine and oats cultures, when washed CO_2 gas was introduced at the bottom of the vessel for 10 minutes, 3 days a week at a pressure of 2.5 atmospheres. In their second series, when water saturated with CO_2 was used, no injury resulted; but even this procedure did not in MITSCHERLICH's experiments (118) prevent injury to the plants.

In none of the experiments on the effect of the artificial addition of CO_2 is the total concentration of carbon dioxide in the soil at any definite interval of time known. The possibility of toxic doses, therefore, is not eliminated. Thus, in PARKER's experiments (140) the concentration of carbon dioxide was sufficient to increase markedly the absorption of silica. Evidence exists that different plants differ in sensitivity to CO_2 (45, 179), although in the culture solution experiments of HALL (56) wilting occurred when CO_2 was aspirated through all culture solutions. STOKLASA (182) and also LUNDEGÅRDH (102, 103) found that a concentration as low as 1 per cent. of CO_2 in the soil atmosphere 15 cm. below the soil surface retarded growth, and was even toxic to some plants.

It is of interest to note here that PFEIFFER and BLANCK (145) argue from the negative results obtained by CO_2 additions in their "oder-sand" cultures in the presence of rock phosphate that the data afford proof that other acids are exuded by roots, because, although many of the plants with which they experimented were able to utilize rock phosphate quite well, the artificial addition of CO_2 gave in all cases decreased yields.

3. ROOT EXCRETIONS OTHER THAN CO_2

a. THE DIVERGENT VIEWS.—The tendency of the European workers (1, 28, 55, 120, 183) and also of some of the American investigators (52, 67, 68) is to emphasize the importance of carbon dioxide exudation, and to regard the rôle of other acid exudations, relatively, of little significance, and, indeed, in some cases (52, 55, 183) to question their existence. Others (28, 120) have claimed to have found substances other than acids in the root exudations.

b. DISTINCTION BETWEEN EXCRETIONS FROM LIVING AND DEAD CELLS.— Although the fact that acids may be excreted from dead cells was early recognized by CZAPEK (28), it may be questioned if, in planning some experiments (12), sufficient attention has been paid to the necessity for making conditions such that the excretions of living and dead cells could be differentiated. That this point is of importance is shown by the results of JOHNSON (75), who found marked differences in the quantities of Ca^+ and Cl^- absorbed from a $CaCl_2$ solution, in the case of live and dead beet cells. The dead beet cells removed only 26.2 per cent. Cl from the roots whereas living beet cells removed 43.7 per cent.; but the proportion of Ca absorbed was nearly the same in both cases.

OSTERHOUT (133) and also RUDOLFS (160, 161) have also called attention to the effect of hydrolytic dissociation of neutral salts and to the effect of the differential rate of ionic absorption. These secondary effects, due to differences in the permeability of living and dead cells, will be discussed later in this paper.

c. ROOT SAP ACIDITY AS A BASIS FOR CHEMICAL METHODS OF DETERMINING NUTRIENT REQUIREMENTS.—(1) *The direct method of approach.*—The assumption that acids were exuded from roots served as a basis for the attempts of DYER (38) and his school to ascertain the fertilizer requirements of soils. DYER argued that if acids are exuded, the total acidity of the sap should be an approximate measure of its solvent action. The sap acidity was obtained by averaging the results of determinations for total acidity of the water extracts of the roots from over a hundred different species of plants. This was found to be equal approximately to a 1 per cent. solution of citric acid. LEMMERMANN (96) also adopted a similar criterion and, as we shall see later, the greater assimilatory ability of the Leguminosae compared with the Gramineae was attributed by him to the greater total acidity of the root sap of the former.

Information on the nature of the root exudations was also sought by CZAPEK (28) and KUNZE (89) by bringing intact seedling roots in contact with litmus paper and noting the change in color. It has been suggested by HAAS (54) and others, however, that it is not possible to reach definite

conclusions from this type of experiment because different roots may differ in their capacity to absorb the blue dye of litmus. Differential absorption cannot account for all the facts, for KUNZE (89) showed by titration in parallel experiments that the acidity exhibited was far greater than that indicated by the turning point of litmus. And there is no evidence in KUNZE's experiments that the acids arise from injured or dead cells. It is clear, too, that KUNZE recognized the limitations of litmus paper as an indicator, for he states that in some plants the amount of acid lies below the sensitivity of litmus.

CZAPEK (28) attributed the red color obtained by pressing seedlings against blue litmus paper to the exudation of KH_2PO_4, and emphasized the fact that the exudation of this salt could not be explained by any injury to the cells of the root hairs because the same result was obtained in culture experiments where there could be no question of injury; but it is to be noted that CZAPEK did not find the drops of liquid—always to be observed on the roots of seedlings developed in a humid atmosphere—to be acid. PRIANISHNIKOV (153) also found traces of PO_4 in the root secretions of *seedlings, but not from full grown normal plants.*

The whole subject of the secretion of acids other than CO_2 was subjected to a critical investigation by STOKLASA and ERNEST (183) with *Hordeum vulgare* and *Zea mays,* using a different technique. The seedlings were allowed to develop for 15 days in distilled water and then transferred to experimental cylinders, through which 20 liters of sterilized air free from CO_2 were passed. In one series of experiments a mixture of O_2 and N_2 gas was used. Under these conditions CO_2 and a trace of H_2 was found, but no PO_4 nor SO_4. In view of the fact that a large number of investigators (8, 13, 79, 94, 101) had reported the presence of lactic, acetic and formic acids in the root exudations of plants, STOKLASA and ERNEST carried out a similar series of experiments from which they conclude that these acids were only exuded when the root system suffers from lack of oxygen and is the result of partial intramolecular respiration. When the supply of oxygen was sufficient only carbon dioxide was found. These experiments were carried out at a uniform temperature (20–22° C.) and observations were made for 10–20 days. Air containing q-radium emanations increased respiration considerably.

STOKLASA and also HALL (55) have argued, from an ecological standpoint, that it is improbable that such important nutrients as K and P_2O_5 would be exuded from the root system; but this position is untenable, for many experiments (15, 207) show very clearly that plants may return to the soil at some stage of their growth, usually at maturity, some of these important nutrient elements.

(2) *The indirect method of approach.*—Some investigators (117, 145, 181, 192), on the other hand, have sought a solution of the problem of acid exudation of roots by determining the assimilatory power of different plants for difficultly soluble materials, like rock phosphate, and HALL (55) has sought a solution by taking out a balance sheet between the acid and bases contained within the completely developed plant.

(a) *Mitscherlich's view.*—The conclusions from the indirect methods devised by MITSCHERLICH (119) and that adopted by PFEIFFER and BLANCK (145) are contradictory. MITSCHERLICH (119) maintains that his "Wirkungsfaktor" values are a constant characteristic for each manure (fertilizer) on all crops and all soils. This "law" of MITSCHERLICH is expressed by the equation

$$\frac{dx}{dy} = k(A - y) \tag{7}$$

or $\log_e (A - y) = \log_e A - c(x + b)$ (8)

where: c = "Wirkungsfaktor" or action produced by the nutrient element applied,

A = maximum yield when x is not limiting,

x = amount of nutrient element added,

b = amount of available nutrient already present in the soil,

y = yield obtained by application of nutrient element x.

He argues, therefore, from the constancy of *c* that all plants must have at their disposal the same agent for the decomposition of difficultly soluble material and that this is CO_2 exclusively. In recent years he appears to have changed his views to some extent as a result of the following experiment (120), which is easily carried out. When a very dilute solution of HCl (0.0001 N) is added to root hairs observed under a microscope, at first local swelling up of the protoplasm can be observed, followed by a rupture and expulsion of the protoplasm into the surrounding medium. After some time *new root hairs are formed,* showing that the injurious effects of too high hydrogen-ion concentration have been overcome. These phenomena may, in part, be explained by the tendency of plants to change the reaction of the medium in the direction of neutrality as observed by PANTANELLI (138), HOAGLAND (64, 65, 67) and THERON (185). The medium might be neutralized by the absorption of hydrogen-ions and the excretion of OH ions simultaneously, or, as MITSCHERLICH believes, the injurious effect of the acid may be counter-balanced by the liberation of substances of the nature of organic buffers. These ideas would coincide with MAZÉ's (109) and also COMBER's view (26) that substances other than those of acid nature take part in the solubilization of mineral nutrients. That considerable organic matter is liberated from the root sap and root hairs sloughed off by plant roots is shown by the observations of LYON and WILSON (105), but

these findings are not applicable as evidence to the exudations of intact root hairs. MINIMA's investigations (116) afford some evidence, although indirect, that organic acids are excreted by roots, on the basis that the period of maximum excretion of non-volatile organic acid buffers is coincident with that of maximum acidification of the soil.

MITSCHERLICH, in his earlier papers (118), held the view (146, 147) that the amount of P_2O_5 absorbed from rock phosphate by oats was identical with the amount dissolved by a saturated solution of carbon dioxide, and concluded (117) that the different "Aufschliessungs-vermögen" of different plants could be explained by differences exhibited in their ability to develop root systems, which, in turn, is proportional to the amount of carbon dioxide respired by them. More recently he has modified these views and has discarded chemical methods as being inadequate (119). Indeed, it has been found by numerous workers, especially by KÖNIG (82) and SCHLOESING (168), that oats and other plants may absorb far more P_2O_5 from rock phosphate and other soil phosphates than can be dissolved by a saturated solution of CO_2. FREAR and ERB (43) also drew similar conclusions with respect to the soil K_2O of the fertilizer plots of this Experiment Station.

In evaluating these opposing claims, it should be remembered that STOKLASA (180) and STOKLASA and ERNEST (183) found that lactic, acetic, formic and oxalic acids were excreted only when the root system suffers from lack of oxygen; but as yet there are no critical experiments indicating whether or not under average field conditions, the system in immediate contact with the roots may not be relatively deficient in oxygen. The accumulation of these organic acids due to incomplete oxidation of the products of respiration has been found by STOKLASA to be highly toxic to plants. This view is further supported by MAZÉ's experiments (109) with peas in sterile cultures. That the problem of air renewal is a serious problem, especially in some of the finer textured soils, is apparent from technical remedies such as drainage and crop residues devised to meet it.

(b) *Hall and Miller's experiments.*—One of the arguments used by HALL (55) upon which to substantiate his conclusions that only CO_2 is excreted by the plant is based on the findings of HALL and MILLER (57) that the net action of the plant upon the soil is to leave an excess of base in the soil. This result was arrived at by taking a balance sheet between the acid and bases contained within the completely developed plants (wheat, barley, sweeds and hay) of the Rothamsted rotation experiments on the Agdell field, and also of wheat and cabbage plants grown in culture solutions. These experiments, however, do not take into consideration the effect of hydrogen-ion concentration. As THERON (185) and others have empha-

sized, greater equivalent proportions of anions than of cations are absorbed on the acid side and on the alkaline side the reverse holds true. If HALL's statements had been made later, they undoubtedly would have been modified as a result of the recent progress in physical chemistry. Furthermore, the validity of the assumption made by HALL that all the nitrogen in the plant should be calculated as an acid on the basis that all the nitrogen enters the plant as nitrate may be questioned.

(c) *Kappen's experiments on expressed root saps.*—The procedure of KAPPEN (77) of determining the total acidity and hydrogen-ion concentration of the expressed sap of the roots of mature plants grown in field experimental plats is more pertinent; but even in these experiments values cannot be regarded as absolute, because—as we shall see later—of the existence of a hydrogen-ion concentration gradient · (53, 195). Moreover, KAPPEN's experiments are open to the criticism that they do not distinguish between diffusion products and exudations of the roots. Considered, however, from the standpoint of the relative comparisons between total acidity and hydrogen-ion concentration of the respective root saps, KAPPEN's data indicating low hydrogen-ion concentration values but high titration numbers are instructive. Only 0.1 per cent. of the hydrogen-ions were found to be present in the ionic condition, the remainder being in the undissociated form. The inevitable conclusion is that the root saps must consist of a mixture of free organic acids and their alkali or alkali-earth salts. This point is of interest because such an arrangement would ensure the organism against too violent fluctuations in the degree of acidity of its sap, and is analagous to the conditions that exist with other physiological fluids, blood, urine, etc. Both GROH (49) and PAVLINOVA (143) come to the same conclusion as KAPPEN, but the deductions of the latter, unfortunately, are of limited application because only seedlings were used. They are of interest, however, in that they show that the older technique adopted by CZAPEK (28) is useless to determine the reaction of the water exuded by guttation.

(d) *Haas's experiments.*—HAAS (52) sought a solution of the problem of root exudation by determining simultaneously the hydrogen-ion concentration of solutions in which seedling plants were growing and also in controls without plants. After the CO_2 had been expelled by a current of pure H_2, the hydrogen-ion concentration of both series of cultures was identical. Although this method eliminates the possibility of confusing the exudation of living cells from those of dead cells, it is doubtful if the conclusion drawn by HAAS from these experiments, *viz.*, that plants do not exude other acids than CO_2, can be extended to *mature plants*. The mineral nutrient requirements of the 10 day seedlings with which HAAS worked are, of course, much less than in more mature plants.

C. The influence of the degree of the acidity of the cell sap

In view of the emphasis placed by many investigators upon the influence of the degree of the sap acidity of cells as a determining factor in the feeding of plants, it is pertinent for us to inquire more closely into this phenomenon.

1. Does a hydrogen-ion concentration gradient exist

Although there is sufficient evidence to indicate that the hydrogen-ion concentration of the substrate has an indirect influence on the growth of plants, it is not definitely known what, if any, relation exists between the hydrogen-ion concentration of the medium and that of the metabolically active tissues. JACOBS (73, 74), in the case of isolated cells, found none. HOAGLAND's results (66) on barley grown in water, in sand, and also in soil cultures indicate that the pH values of the sap of the tops were almost identical, even though the pH of the nutrient media varied widely, *viz.*, from 4.94 to 6.76 in water cultures and from 7.03 to 7.34 in soils. REED and HAAS (157, 158) report similar results from water culture experiments. The latter (157) and also THERON (185) have obtained comparable results in the case of the sap of tops, but in the roots THERON (185) noted that the pH increased with increase in pH of the medium (substrate). The existence of a definite hydrogen-ion concentration gradient in the case of algae has been shown by CHILD (23) and in some of the higher plants, grown in soils, by HAAS (53) and in animal tissues by GUSTAFSON (51). The regulatory influence of buffer systems, therefore, must vary considerably in different parts of the plant. The data of HAAS (53) are illuminating. In *Melilotus alba* (second year growth), the following pH values were obtained: Soil extract, 7.68; root (6" portion below upper 2"), 5.82; root (2" of upper part), 6.46; leaves and petioles, 7.04; stems, 6.68. The pH of the mixed upper 3" of the tops, stems, leaves and buds was 8.0. As REED and HAAS (158) later pointed out, any hypothesis based upon differences of acidity of the plant sap and the external medium must account not only for the accumulation of inorganic elements within the roots but also for their movements to other portions of the plant. Investigators in this field must, moreover, take cognizance of the fact that marked change in the hydrogen-ion concentration usually occurs within an hour after expression of the plant sap (25, 50, 61, 195).

From the foregoing, it is apparent that we are not yet in possession of sufficient evidence to determine whether or not the cell sap of tissues possessing similar functions have hydrogen-ion concentration values that may be characteristic of the species. The assumption that root sap acidity values are a characteristic of the species formed the basis of much of the experimental work by the German investigators, (1, 89, 96) in their search

for a solution of the causative factors determining the "Aufschliessungs-vermögen" of different species. Thus, the Gramineae and the Leguminosae were supposed to be sharply differentiated (96) on the basis of the acidity of their root sap, until KAPPEN (77) and later STOKLASA (181) showed very definitely that the distinction held neither for the titration nor hydrogen-ion concentration values. STOKLASA's experiments (181), if confirmed, would lead to the conclusion that the acidity of the sap of the roots of all cultivated plants is near neutrality, except when insufficient oxygen is present, under which latter condition the acidity increases. He emphasizes the significance of this fact for the development of the bacteria of the rhizosphere.

As we shall presently see, working on this assumption, TRUOG (194) considers that the feeding power of different plant species is a function of the different intensities of the acidity of the respective saps. Thus, buckwheat and clover are cited as types of plants with high and low acidities, respectively. The table below, however, taken from the data of TRUOG and MEACHAM (195), KAPPEN (77), HAAS (53), STOKLASA (181), and ABERSON (1), will show how impossible is such a classification, at least with the methods hitherto adopted.

TABLE III

pH OF BUCKWHEAT AND RED CLOVER ROOTS REPORTED BY SEVERAL INVESTIGATORS

INVESTIGATOR	BUCKWHEAT	RED CLOVER
TRUOG and MEACHAM	4.0 – 4.03	5.6 – 5.9
KAPPEN	4.9 – 5.3	6.2
ABERSON	5.7 – 5.9	7.9 – 8.4
HAAS	4.8 (mature plant) 5.4 – 5.9 (seedlings)	5.8 – 6.1
STOKLASA	6.2	6.6

Further development of NĚMEC's work (125) on the hydrogen-ion concentration of the sap of seeds of a large number of different species of plants promises—from the practical standpoint—to clarify the whole situation. Seeds are more nearly constant in composition than other parts of plants, and NĚMEC found that the hydrogen-ion concentrations of the seeds of plants adapted to acid soils are relatively much higher than those adapted to alkaline soils. It is of interest to note that the saps from the seeds of barley, corn and peas were found to have the lowest hydrogen-ion concentration, pH 6.45 – 6.30.

It has been a matter of long observation that such plants as sweet clover, alfalfa and lettuce are adapted to grow on soils at or near the neutral point.

When soils to which these plants are adapted are allowed to go unlimed, they are replaced by others that are less sensitive to acid conditions. CAMARGO *et al.* (19) have recently shown in controlled experiments that the optimum growth of roots of the coffee plant is at pH 4.2, which affords an explanation of their adaptability to acid soils. The coffee plant is greatly injured by even moderate liming.

D. Theories proposed to explain differences in the feeding power of plants

1. THE APPLICATION OF THE LAW OF MASS ACTION

TSCHIRIKOW (196, 197) stressed the importance of the CaO/P_2O_5 ratio in the plant as an important factor in the ability of different plants to supply themselves with enough P_2O_5 from difficultly soluble phosphates. Plants having a high energy absorption for Ca would, according to him, depress the Ca concentration of the soil solution, resulting in an increased solubility of the phosphate. The smaller the amount of P_2O_5 necessary for the formation of one unit of organic substance of the plant (*i.e.*, the smaller the percentage of P_2O_5 in the plant), the greater obviously would be the effect of P_2O_5. From this viewpoint the capacity of the plant to absorb difficultly soluble phosphates is directly parallel to the quantity of Ca absorbed and inversely parallel to the P_2O_5 requirement of the plant.

TRUOG (192, 194) has extended TSCHIRIKOW's views further and has invoked the concept of equilibrium between calcium phosphate and carbonic acid, on the one hand, and the products of their interaction, on the other, as expressed by the equation:

$$Ca_3(PO_4)_2 + 2H_2CO_3 \rightleftarrows Ca_2H_2(PO_4)_2 + CaH_2(CO_3)_2 \qquad (9)$$
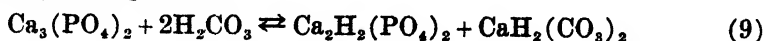
He contends that plants such as buckwheat, which the majority of culture experiments show is capable of utilizing the phosphorus of rock phosphate in large measure, are supposed to be able to absorb both of the products to the right of the equation. It is to be inferred, moreover, as already stated (p. 462), from TRUOG's later expansion of this view that the rate of absorption is determined by the degree of acidity of the saps of these plants. Thus, buckwheat is characterized as a plant with "high internal acidity." In the case of plants having a low degree of sap acidity, one of the products to the right of the equation (calcium bicarbonate) is assumed to be incapable of absorption by the plant at a rate comparable with that of the other product (di-calcium phosphate); consequently, the operation of the reverse reaction will prevent the further solution of the rock phosphate. Corn and oats are placed in this class by TRUOG.

This view has been supported (6, 7, 53) and as vigorously disputed (30). In order to clarify our ideas, it should at the outset be remembered

that, although the possibility (p. 456) of absorption of salts in the undissociated form (133, 135, 137) must be considered possible under some conditions, e.g., in the case of detached cells, the weight of evidence (67) is that absorption in normal intact plants takes place in the ionic condition. Now there is ample evidence, as we shall see later, to show that not only do root hairs of different species differ in their degree of permeability to the same ion, but that the different relative velocities of the anion and cation of a salt may also be a factor in determining the equilibrium. We must then be prepared to consider ionic equilibrium, although the existence of such equilibria between the ions in the cells and the ions in solution has not as yet been established definitely.

2. EVIDENCE FOR AND AGAINST TRUOG'S VIEWS

a. BAUER'S SAND CULTURE EXPERIMENTS.—The results of BAUER (6) have been cited in support of TRUOG's views. In these experiments corn was grown in sand cultures treated with rock phosphate (or acid phosphate) as the source of P, and $NaNO_3$ or $(NH_4)_2NO_3$ as the source of N; but the analytical data are insufficient to show that the improvement in growth in the leached pots was due to removal of calcium as the bicarbonate. Removal of toxic substances by leaching must also be considered a factor (119). Further critical experiments on this point should include pots without plants as controls.

b. THE VIEWS OF THE CALIFORNIA GROUP.—DAVIS, HOAGLAND, and LIPMAN (30) cite NEWTON's (126) results with peas and barley in water culture solutions, which grew normally without bicarbonates, and, moreover, in solutions at hydrogen-ion concentration at which bicarbonate ions could not exist to any extent. But this argument may be weakened by the fact that HOAGLAND (67) and others have later deduced evidence to show that rapid replacement of $-HCO_3$ ions by other ions may occur.

REED and HAAS (157) have also argued against TRUOG's view on the basis of their experimental work with walnut and orange trees. In experiments with the former the effect of a solution of $Ca(OH)_2$ of pH 9.0 was determined. Although the percentages of Ca in the ash of the tops and roots were 14.60 and 9.80, respectively, the pH of their sap (5.26–5.48) showed no significant changes from values obtained when such plants were grown in complete culture solutions. This same argument, however, used by REED and HAAS to refute the existence of a relation (193) between the absorption of calcium and the degree of acidity of the sap, breaks down completely when certain experimental data (78) may be interpreted, by the same method of reasoning, to prove that calcium may be necessary for precipitating organic acids formed as a by-product, on the basis that

mottled leaves of citrus trees starved of calcium are considerably more acid and contain distinctly less calcium than normal leaves.

Again, REED and HAAS (158) are inclined to the belief that there is no evidence in support of the assumption by TRUOG (194) that absorbed materials are converted into insoluble compounds as rapidly as they accumulate in the plant, or that absorption *necessarily depends* on the precipitation of ions within the plant. The italics are the writer's. But this conclusion is not justified from the experimental data supplied. Thus, REED and HAAS (158) find that in sand cultures the concentration of water-soluble K increases in the ash of leaves, trunk, shoots and stems, and that the concentration of soluble Ca increases in the leaves *but decreases* in the shoots, trunk and roots as the concentration of the nutrient solution increases. In soil cultures 45.82 per cent. of the Ca was soluble in the leaves, 15.03 per cent. in the shoots, and only 7–9.5 per cent. in the trunk, roots and rootlets. In buckwheat nearly all the calcium is insoluble in water (67). The fact that *Nitella* (69) contains large quantities of soluble potassium and calcium salts has also been used as an argument (30) against TRUOG's views. But to what extent the extension of data obtained from such abnormal structures as *Nitella* to the higher cultivated plants is justifiable remains to be determined.

The work of KOSTYCHEV and BERG (88) has an important bearing in this connection. They have determined the forms in which Ca is present in the tissues of a number of plants. The following interesting data, table IV, are taken from their paper:—

TABLE IV

FORMS OF CALCIUM IN PLANTS

	PERCENTAGE OF THE TOTAL CA		
PLANT	SOLUBLE IN WATER	COMBINED WITH PO_4 AND HCO_3	CA OXALATE
Trifolium repens (leaves)	42.4	38.6	19.0
Alchemilla sp. (leaves)	35.6	40.3	24.1
Caragana arborescens (leaves)	21.0	23.1	55.9
Solanum tuberosum (leaves)	16.5	34.5	49.0
Lemna minor	11.6	45.7	42.6

In apple tree experiments (190) the writer has found in mature leaves relatively low percentages of the total Ca soluble in water, *viz.*, 17.2 per cent., and relatively high percentages as the oxalate, *viz.*, 54.3 per cent. The above data might, therefore, be just as readily used to support TRUOG's views as to refute them.

CHIBNALL (22) has recently shown that one-tenth of the water-insoluble calcium of cabbage leaves consists of calcium phosphatide.

c. SOME WEAK POINTS IN TRUOG'S THEORY.—

(1) *The experiments of Parker—Bauer—Bartholomew.*—According to TRUOG (194) plants with a slightly acid or neutral sap, like sweet clover and alfalfa, are more able to utilize the potassium advantageously from lower concentrations of the element than plants, like buckwheat, having a more acid sap; but PARKER (139) found that buckwheat absorbed relatively large quantities of soil potassium. Moreover, BAUER (6) found that both buckwheat and sweet clover readily absorbed calcium from rock phosphate. BARTHOLOMEW (4) also failed to find any relation between the amount (percentage) of calcium absorbed and the ability of these plants to feed on rock phosphate, although the substrate and nutrient medium were the same as that used by TRUOG (quartz sand).

(2) *The effect of the destruction of the buffer systems.*—No account has been taken of the buffer systems present, the destruction of which must be of greater influence than the factors already noted. The buffer action of calcium bicarbonate, for example, is a function of the hydrogen-ion concentration of the medium, and is readily destroyed if the hydrogen-ion concentration varies from pH 7.5.

$$Ca(HCO_3)_2 + NaOH = CaCO_3 + NaHCO_3 + H_2O \qquad (10)$$

$$Ca(HCO_3)_2 + H_2SO_4 = CaSO_4 + 2H_2O \quad + 2CO_2 \qquad (11)$$
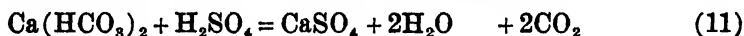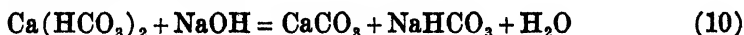
The destruction of $Ca(HCO_3)_2$ results, as shown by BUTKEWITSCH (16), in increased yields—in some cases as much as three times—and the amount of calcium in the medium decreases. The stronger the buffer action the greater the CaO/P_2O_5 ratio. From this standpoint, the inhibitory action of $Ca(HCO_3)_2$ on phosphate utilization may be regarded as that of paralyzing the root activity of the plant. *In this sense and in this sense only may TRUOG's view hold.*

(3) *Do the terms "high" and "low" acidity have any meaning?*—The validity of TRUOG's conception must necessarily rest also upon the accuracy of his classification of the relative acidities of the saps of plants into high, medium and low; but, unfortunately, we have no definite knowledge of the relative acidities of plant saps. As we have seen, the variation in different parts of the same plant is so great that it is not, as yet, justifiable to use the terms "high" and "low" in classifying plant saps. The hydrogen-ion concentration of the sap of seeds is much more constant; therefore, in any attempt to correlate hydrogen-ion concentration of plant tissues with nutrient supply, the examination of the sap of seeds would be the most profitable line of attack.

d. LACK OF HARMONY OF OBSERVATION WITH THEORY.—Nevertheless, it is highly improbable that the results upon which PARKER and TRUOG (**142**) based their classification of plants into (1) high Ca and high N, and (2) low Ca and low N are accidental, as has been contended (**129**). On the contrary, the preliminary studies of STORCK and RIPPEL (**134**) support the existence of such a relationship between Ca and N. True, the analytical data presented by PARKER and TRUOG represent the composition of plants grown under very varied and different conditions. But an examination of the paper (**142**) will show at once that the data have been collected with great care and, moreover, TRUOG has himself conducted carefully controlled experiments. He recognizes (**193**) that isolated analyses may be found that are relatively quite different from those used—especially of plants that have reached maturity or were cut as hay—due to losses of Ca, in part possibly by leaching (**95**).

If we accept this relationship between calcium and nitrogen as an actual fact, to what may it be attributed? Surely not to acids arising from the decomposition of proteins, as TRUOG assumes. The older physiologists (**33**) considered that "living" protein substance of the protoplasm functions as the immediate material for respiration, resulting in the production of the acids found in plants. But, as pointed out by KOSTYCHEV (**87**), this assumption contradicts an abundance of facts that have been established recently without question—that sugars are much more easily oxidized than proteins. Most of the plant acids arise from the carbohydrates and fats that serve as the fundamental sources of energy of respiration (**156**). As a matter of fact the amount of energy obtained by the decomposition of proteins is very small, and, moreover, the amino acids formed are readily converted by the plant again into proteins (**176**). Only after the complete consumption of sugars do plants begin to burn the protein material of their protoplasmic framework, and then the CO_2/O_2 ratio falls to 0.70–0.80.

The explanation of the Ca/N relations observed by PARKER and TRUOG (**142**) is more probably to be explained by the antagonistic action of Ca and K resulting in an increased absorption of N and P_2O_5 (**188**).

e. EXTENT TO WHICH BASES FUNCTION AS NEUTRALIZERS OF PLANT ACIDS.— TRUOG and MEACHAM (**195**) and also HAAS (**53**) have examined the hydrogen-ion concentrations of the tops of a large number of plants grown both in limed and unlimed plots, and, without considering the possibility that the results are within the limits of experimental error (**185**), it was found that with few exceptions the pH of the tops of the *limed* plants was higher than that of the *unlimed*. It might be argued from these facts (**53**) that one function of calcium is to adjust the pH of the soil solution so as to

enable the plant to secure a sufficiently rapid supply of bases to neutralize the injurious effect of too high acidity; but, apart from the fact that neither CLEVENGER (**25**) nor DUSTMAN (**37**) have been able to corroborate such a conclusion, there is absolutely no justification to assume that this function is more peculiar to calcium than the other bases, *e.g.*, magnesium and potassium (**90, 91**). Moreover, it is not necessary to assume that the disappearance of acids is accomplished solely by the mechanism of neutralization, since ASTRUC (**2**) has shown conclusively that the disappearance of acids—which are always higher in the young plants—is due not so much to their "neutralization" as to the process of respiration and esterification.

f. CALCIUM REQUIREMENTS OF PLANTS ARE VERY DIFFERENT.—It is certain that plants cannot continue growth without calcium and, although different species respond differently to its presence, injury does not result if sufficient quantity of soluble salts are present in the media (**157**). The observation of LOEW (**114**), therefore, of the death of cells on treatment with salts that precipitate Ca must be interpreted accordingly.

The response of species to varying quantities of calcium appears to be an important ecological factor (**201**). Thus, SKEEN (**175**) has shown that *Phaseolus vulgaris nanus* must have large amounts of calcium and that *Lupinus albus* obtains its maximum growth with relative traces.

From the foregoing, we are forced to conclude that, although the suggested explanation (p. 467) offered to account for this Ca/N relationship may not explain all the facts, there is no sound reason for denying the existence of this relationship. It is unfortunate, from this standpoint, that the nitrogen content of the oat plants grown by SHEDD (**171**) on different soils and which showed such extreme ranges in calcium content are not known. This would have given us some valuable information.

3. CONCLUSIONS FROM RECENT MORE DIRECT EXPERIMENTS

RUSSELL (**162**) considers that the critical problem is to know whether the concentration of the substrate (nutrient solution) with respect to calcium has decreased to a greater extent in the "high feeding" plants than in the "low feeders." Such experiments are at present being carried out by DOMONTOVITSCH and SCHESTAKOW (**34**), of which only the preliminary results have been published to date. In sand culture experiments with lupines, buckwheat, mustard, millet and oats—using rock phosphate (size of particles 0.1 mm.) as the source of P—the growth of the plants and concentration with respect to P_2O_5 and Ca were compared between pots containing mixed plants, *viz.*, (1) buckwheat and oats, (2) lupines and oats, (3) lupines and millet, (4) mustard and oats, and pots containing an equal number of plants grown alone. The following table V gives the results of the preliminary work:—

TABLE V

DATA FROM DOMONTOVITSCH AND SCHESTAKOW

NR. DER GEFÄSSE	P_2O_5—QUELLE UND PFLANZENART	pH	P_2O_5 IN 100 CC. DER LÖSUNG MG.	CA IN 100 CC. DER LÖSUNG MG.
4	Rohphosphat Hafer	7.43	0.020	95.0
16	" Hirse	7.14	0.018	111.93
6	" Buchweizen mit Hafer	5.43	0.041	....
5	" Buchweizen	6.25	0.036	....
11	" Lupine	5.34	0.767	30.85
12	" Lupine mit Hafer	5.17	0.679	75.89
17	" Lupine mit Hafer	4.80	1.350	48.59
26	" Hafer	7.21	Spuren	....
36	" Senf mit Hafer	6.35	{ grössere Spuren	....

The growth of oats and millet was scanty and not much greater than those plants without any phosphorus addition. In the mixed cultures with lupines the yield of oats and millet was increased 545 and 846 per cent. respectively, over the yields of these plants grown alone. Mustard and buckwheat also influenced the growth of these Gramineae to a remarkable extent. Unfortunately, calcium determinations are not reported in all cases; but it is evident that the mixed cultures with lupines have reduced the concentration of the calcium under that in the pots in which the Gramineae grew alone. The hydrogen-ion concentration of the medium in the mixed cultures is also much lower than that in the cultures of plants grown alone. These results indicate that the differences in the utilization of rock phosphate in these experiments are to be correlated with their effect on the hydrogen-ion concentration of the root medium and, perhaps, also with the unequal rates of absorption of calcium. The data for concentration of P_2O_5 show no relation between the rates of absorption of phosphate ion by the different plants.

HOAGLAND has recently reported that with the same source of rock phosphate, buckwheat was able to make normal growth, whereas tomato plants were almost entirely unable to obtain phosphate from this source—yet the tomato plants had a much higher percentage of calcium than the buckwheat plants (68). The conclusion is drawn that the solid phase of the soil and its ability to keep the soil solution supplied with calcium is an important factor. Results obtained by MAZÉ (109) may be similarly interpreted. It would be of interest for investigators in this country to conduct experiments similar to those of DOMONTOVITSCH and SCHESTAKOW's work and, in

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addition, to determine the content of the plants in Ca and PO_4. It is well to bear in mind, however, the possibility that the carbon dioxide exuded by roots may, under the artificial conditions of many of the experiments reported, be of far greater importance than under normal conditions and for this reason it is undoubtedly more advantageous to use soils as the substrate.

E. The influence of the extent of the root system

1. Is THERE ANY RELATIONSHIP BETWEEN FEEDING POWER AND EXTENT OF ROOTS

That great differences exist with respect to the extent of the root system among different types of plants is a common observation. A review of the literature has been given by MILLER (115). This difference in the growth of plant roots outwards, together with the differences in their respiration energy, is regarded by some authorities (30) as an important factor in the mechanism of absorption of ions by plants.

TRUOG has also clearly recognized that differences in the extent of root system may be a factor in the absorbing power of plants. This is quite evident from a statement in one of his earlier papers (193) to the effect that "the feeding power of a plant for lime depends largely on the extent and character of the root system." From the results of experiments carried out later, TRUOG modified this view and, as we have seen, stressed the internal acidity of the sap as a more important factor in plants like buckwheat, which have a very restricted root system.

But there are several experiments (84, 152, 154, 155, 192) that indicate no evidence of the existence of any relationship between feeding power and the extent of the root system. In quartz sand cultures with rock phosphate as the source of phosphorus (84, 152, 154, 155, 192) much larger yields and a much higher percentage of P_2O_5 were obtained in the case of the legumes (peas and lupines) and also of buckwheat than of the cereals. In other words, in these experiments buckwheat—with a small root system—(145, 146) utilizes rock phosphate better than the cereals which have, relatively, a very extensive root system. PARKER's data (139) are of interest in this connection. Comparing the absorption of sorghum and cotton under similar conditions, it was found that the latter absorbed more than double the amount of ash constituents than the former. If, as the majority of field observations tend to indicate, sorghum has a more extensive root system than the tap-rooted cotton plants, differences in absorption of mineral constituents cannot, in PARKER's experiments, be attributed to differences in extent of root systems. On the other hand, DAVIS et al. (30) cite NEWTON's experiments (126) to show that the extent of root system is an important factor in the absorption of ions from soil. This conclusion is based on

NEWTON's experiments in which peas and barley were grown together. Under these conditions the peas absorbed more calcium than barley from *soil cultures*; but there was no similar difference in *solution cultures*. It is argued, therefore, that if barley and peas differed considerably in extent of root system such results would be expected to follow. Data on the growth of the respective root systems in these experiments are not given. In a later experiment (128) on the evolution of CO_2 from barley and peas, the dry weights of the roots of peas were found to be less than those of barley. NEWTON himself (128), however, is inclined to attribute these differences to the much larger respiration energy of peas over barley than to differences in the extent of root systems. But HOAGLAND (67) later pointed out that comparisons of extent of root systems have only a very limited value, because the general appearance and size of a root system is not necessarily an accurate index of the total active absorbing area. In this connection it is of interest to note that RUSSELL (162) is of the opinion that the whole question of the supply of nutrients involves both root spread and replacement of ions, although no experimental evidence is cited.

Consequently, views on the relation of extent of root system to feeding power are necessarily speculative until more critical data are forthcoming. The method of determining the actual absorbing surface such as that employed by DUSTMAN (37) and others is unquestionably the most accurate. A critical discussion of the relative merits of the various procedures has been given recently by WEAVER and HIMMEL (202).

F. The influence of other factors

1. PERMEABILITY

In an earlier paper (187) the writer has discussed some of the factors involved in "membrane" permeability. Until sufficient quantitative and qualitative data, obtained under rigid control of all the variables are available it is improbable that views on the subject can be anything but speculative. Possibly, a satisfactory working hypothesis may be formulated only when data, based upon cataphoresis experiments, have accumulated from a wide variety of materials carrying no charge (99). The importance of securing more qualitative information is indicated by the interesting properties of the phosphatide $(RO_2 \cdot C \cdot CH_2OH(C \cdot R'O_2)CH_2O \cdot PO_3H_2)$ isolated by CHANNON and CHIBNALL (22). It forms a fat-soluble calcium salt but a water-soluble sodium salt; and, accordingly, cell permeability could be conceived to be altered by a change in the proportion of calcium to sodium salts.

From thermodynamic considerations and also from the kinetic theory it can be deduced that ionic pairs having the higher mobility will pass through a membrane in proportionally the greater quantity, and also that

the permeability of an ion is increased by the introduction of another electrolyte possessing a common ion. Thus, many investigators (100) have found that the absorption of K from KCl is increased by the addition of NaCl; and the effect of the NaCl has been shown to be dependent on the hydrogen-ion concentration of the medium. Since hydrogen and hydroxyl ions have the greatest velocity, the addition of acids (decrease of pH) will increase the diffusion of anions; the addition of alkalies (increase of pH) that of cations (46, 47, 48).

The influence of the hydrogen-ion concentration of the medium on the absorption of *weak acids and bases and their salts* by living cells has been investigated by JACOBS (73, 74). The pH of the medium to which a cell is exposed was found to bear no necessary relation to the pH produced by that medium within the cell itself. The higher permeability of living membranes to undissociated acids and bases results in a higher physiological activity of weak acids and bases compared with stronger ones. Conditions favoring the formation of undissociated molecules, therefore, will promote their penetration into membranes to which they are permeable. The result of these influences may, as LOEB (100) has shown, be to modify the properties of the cell wall in such a way as to accelerate the rate of diffusion of certain ions and retard the rate of diffusion of other ions. $CaCl_2$ and NaCl are important factors in causing such selective diffusion.

From the practical standpoint it is interesting to note that STOKLASA (183) finds that different plants have different selective abilities towards cations and anions, respectively. The Gramineae, in his experiments, absorb anions more than cations; potatoes and beets more cations than anions; legumes anions and cations equally. Of course, further confirmation of such results is necessary.

2. ION ANTAGONISM

There is sufficient evidence to show that Ca and Mg, which precipitate sols, cause contraction and impermeability and that K and Na, which stabilize sols, cause relaxation on the one hand, and greater permeability on the other. The antagonistic effects between univalent and divalent cations in colloidal systems have been studied by MEULEN and RIEMAN (113, 159), by WEISER (203), and also by HÖBER (71). SIMMS (173) has derived a mathematical expression for the antagonism between Na (or K) and Mg (or Ca) in true (non-colloidal) solutions of oxalate. One might expect—although it has not been demonstrated—that the activity of proteins would show a similar antagonism.

It is essential, however, for the investigators to keep in mind the fact that the effect of a mixture of two or more cations (or anions) on cell permeability may be quite different from the effect of one alone when the activity of the ions is due to different combining proportions of the cations

(or anions) in a mixture (such as the soil solution), in proportion to the relative combinations in solutions of each alone (174). Some of the practical agricultural applications of ion antagonism between Ca and K have been investigated by LIPMAN et al. (98), by McCOOL and WELDON (106) and by FONDER (42).

3. GIBBS-DONNAN DISTRIBUTION LAW

In systems containing a non-diffusible ion, a marked effect is observed on the unequal distribution of inorganic electrolytes on either side of the cell membrane. A non-diffusible electro-negative ion will produce a greater concentration of diffusible electro-positive ions and a lower concentration of electro-negative ions on its own side of the membrane than on the other. A non-diffusible electro-positive ion will produce the opposite effect. At equilibrium the product of the concentrations of each pair of oppositely charged diffusible ions is the same on either side (35, 36). The distribution of an electrolyte in such a system will be expressed by the equation:

$$\frac{x_i \, y_i}{v_i^2} = \frac{x_e \, y_e}{v_e^2} \tag{12}$$

where x_i and y_i represent the amounts of cation and anion, respectively, within the membrane at equilibrium; and x_e and y_e the equilibrium amounts of the same pairs of ions in the external solution; v_i and v_e the volume of the solvent on either side of the membrane.

The importance that the GIBBS-DONNAN distribution law may have in the equilibrium conditions and consequent selectivity of nutrients by plants has been mentioned by HOAGLAND (67), PIERRE and PARKER (148), and also by BRIGGS and PETRIE (14). It will be recalled (187) that PARKER (141) found that the displaced soil solution from many of the experimental fields in America were too low in inorganic phosphorus to support growth in the nutrient media when the phosphorus in these soil solutions was used as the source of phosphorus. One of the explanations offered by him to explain this anomaly is that a DONNAN membrane equilibrium may exist, resulting in a higher concentration of phosphorus in the solution on the surface of the soil particles than in the soil solution itself. The mechanism involved has already been discussed by the writer (132, 187). But the exact experimental verification of DONNAN's law in the case of specific plant tissue is difficult, for it is impracticable to measure the x_i, y_i, and v_i accurately, since a calculation of the amount of ions absorbed by means of the difference between their concentration before and after equilibrium is reached does not take cognizance of the amounts of these ions already present in the tissue (14). Hence, extension of the methods of OSTERHOUT (135) and of OSTERHOUT and DORCAS (137) on the absorption of CO_2 by *Valonia* do not afford a basis for generalization, for even here the average

concentration of the ions in the cell, as a whole, is not considered but only that in the cell sap.

As BRIGGS and PETRIE (14) point out, in the plant cell we are dealing with a polyphase system. The DONNAN equilibrium applies to free ions only. In the cell there is evidence that some of the ions are present as undissociated salts of proteins or other substances, or again some of the ions may be physically absorbed. We should expect, therefore, that the simple DONNAN equation would not hold in such systems. That this is the case is apparent from the results of BRIGGS and PETRIE's experiments on the absorption of 0.1 M KCl and NH_4Cl from carrot tissues (14) and from WRIGHT's (214) work on the secretion of high amounts of calcium in milk from the relatively low concentration of this element in the blood plasma. The latter finds that both the degree of dissociation of the protein salts and the establishment of a DONNAN equilibrium must be taken into account in explaining the inequalities of the products in the distribution of inorganic elements in living tissues, whereas the former (14) have deduced that the apparent ionic internal product, resulting from the collective effect of all the phases within the tissue, must have a higher value than that of the external media. BRIGGS and PETRIE (14) point out that "the plant cell is not a simple system, composed of a mere membrane enclosing a homogeneous solution, one of the ions of which is indiffusible; there are at least three phases between which the ions may be distributed—the external medium, the cytoplasm, and the vacuoles. Within the cytoplasm also, and perhaps in the cell-sap, there are micelles, or gel-particles, which can constitute yet other phases."

BUTKEWITSCH and BUTKEWITSCH (17, 18) have advanced as a reason for the inequality of the products of the diffusible ions on each side of a membrane in systems containing a non-diffusible ion, that the degree of permeability of the plasma membrane is not the same in both directions. This has been shown to be the case by WERTHEIMER (204, 205) for certain types of membranes. In such a case the equation of membrane equilibrium will contain, besides the concentration product of diffusible ions, a factor corresponding to the velocity of migration of the corresponding ionic pairs. Thus, if a solution, say of KCl, is separated by a membrane permeable in the two directions, we have :—

$$
\begin{array}{ccc}
x' \quad K^+ & \Big| & K^+ \quad x'' \\
y' \quad Cl^- & \Big| & Cl^- \quad y'' \\
& p' - \!\!\to & \\
& \leftarrow\!\!- p'' & \\
\end{array}
$$
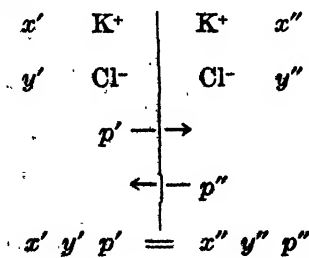
where
$$x' \, y' \, p' = x'' \, y'' \, p''$$

where x and y represent the concentration of K and Cl ions and p a permeability factor.

By this means it can be calculated (17) that in OSTERHOUT's experiments on *Valonia* (134) the permeability of the plasma membrane for Na in one direction is one-fifth that in the opposite direction and in the case of K the difference is 45 times.

Within the limitations already discussed it is possible to interpret the experiments of BUTKEWITSCH and BUTKEWITSCH (17) on the influence of colloidal silicic acid upon the absorption of phosphorus by maize plants by DONNAN's principle. When small amounts of P_2O_5 are present in the nutrient medium, growth and absolute amounts of P_2O_5 increased by the additions of silicic acid in the nutrient medium; but the *percentage* of P_2O_5 remained about the same. It follows that a functional replacement of P_2O_5 by H_2SiO_3 in the plant does not occur, but that the favorable action of H_2SiO_3 on the development of the plants is due to its effect in increasing the absorption of P_2O_5 in a medium that contained minimum amounts of phosphorus for growth (189).

4. THE EFFECT OF DIFFERENCES IN POTENTIAL BETWEEN SOIL AND PLANT

Since the breaking down of carbohydrates sets free electromotive force, part of the energy stored in the plant must be electrical and used in growth. The work of GROH (49) is important. He attributes the different behavior of different plants toward different degrees of hydrogen-ion concentration in the medium to a difference in potential between the plant and soil solutions, which results in a decomposition of the nutrient material. This hypothesis is supported by the following experiment. Peas and also barley were grown in sand and also in soil cultures to which $Ca(NO_3)_2$ were added. During growth individual plants were connected with a galvanometer with one of the platinum electrodes immersed in the soil and the other connected to the plant. The E.M.F. produced varied with conditions—from 0.01 to 0.20 volts. The greater the distance from the roots, of the electrode connected to the plant, the greater the current registered. This shows that the difference of potential existing in the plant itself is much less than that existing between the plant and the soil. The degree of vegetative development apparently was not a large factor in determining the degree of the difference of potentials noted. GROH's work, although of a preliminary nature, is suggestive and should be considered in connection with OSTERHOUT's (136) more refined work on the potential difference in *Valonia* and *Nitella.*

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LITERATURE CITED

1. ABERSON, J. H. Ein Beitrag zur Kenntnis der Natur der Wurzelausscheidungen. Jahrb. wiss. Bot. **47**: 41–56. 1910.

2. ASTRUC, A. Recherches sur l'acidité des végétale. Ann. Sci. Nat. Bot. Ser. 8, **17**: 1–108. 1903.

3. BARAKOV, P. Der Kohlensäuregehalt des Bodens während der verschiedenen Wachstumsperioden der Pflanzen. Russ. Jour. Expt. Landw. **11**: 321–343. 1910. (German resumé.)

4. BARTHOLOMEW, R. P. The unavailability of phosphorus in rock phosphate to some southern crops. Jour. Amer. Soc. Agron. **20**: 913–920. 1928.

5. ————, and JANSSEN, GEORGE. The relation between concentrations of potassium in culture solutions and optimum plant growth. Soil Sci. **27**: 189–203. 1929.

6. BAUER, F. C. The effect of leaching on the availability of rock phosphate to corn. Soil Sci. **9**: 235–251. 1920.

7. ————, and HAAS, A. R. C. The effect of lime, leaching, form of phosphate and nitrogen salt on plant and soil acidity, and the relation of these to the feeding power of the plant. Soil Sci. **13**: 461–479. 1922.

8. BECQUEREL, H. Ueber die Anwendung der electrochemischen Kräfte auf die Pflanzen-Physiologie. Ann. d. Chem. (Liebig) **8**: 92–109. 1833.

9. BIZZELL, J. A., and LYON, T. L. The effect of certain factors on the carbon-dioxide content of soil air. Jour. Amer. Soc. Agron. **10**: 97–112. 1918.

10. BOGDANOV, S. Plodorodie pochvy po novieishim dannym. Selsk. Khoz. i Liesov. **211**: 249–284. 1903.

11. BREAZEALE, J. F., and BURGESS, P. S. The availability of phosphates in calcareous or alkaline soils. Arizona Agr. Exp. Sta. Tech. Bull. **10**: 209–237. 1926.

12. ————, and LeClerc, J. A. The growth of wheat seedlings as affected by acid or alkaline conditions. U. S. Dept. Agr. Bur. Chem. Bull. **149**: 1–18. 1912.

13. BREFELD, OSCAR. Ueber Gährung. III. Vorkommen und Verbreitung der Alkoholgährung im Pflanzenreiche. Landw. Jahrb. **5**: 281–341. 1876.

14. BRIGGS, G. E., and PETRIE, A. H. K. On the application of the DONNAN equilibrium to the ionic relations of plant tissues. Biochem. Jour. **22**: 1071–1082. 1928.

15. Burd, John S. Rate of absorption of soil constituents at successive stages of plant growth. Jour. Agr. Res. **18**: 51–72. 1919.

16. Butkewitsch, W. W. Ueber die Faktoren, welche das Verhalten der Pflanzen gegen schwerlösliche Ca-Phosphate beeinflussen. Landw. Jahrb. **66**: 947–992. 1927.

17. Butkewitsch, Wl. S., and Butkewitsch, W. W. Zur Frage nach der Rolle des "Donnanschen Membrangleichgewichts" bei osmotischen Vorgängen in lebenden Zellen. Biochem. Zeitschr. **161**: 468–487. 1925.

18. ——————, and ——————. Über Wechselwirkung von Ionen bei Diffusions-vorgängen. Biochem. Zeitschr. **204**: 303–321. 1929.

19. Camargo, Theodureto de, Bolliger, R., and Mello, Paulo Correa de. Sur l'influence de la concentration en ions hydrogène du milieu de culture sur le developpement du cafeier. Compt. Rend. Acad. Sci. (Paris) **188**: 878–880. 1929.

20. Cameron, F. K., and Hurst, L. A. The action of water and saline solutions upon certain slightly soluble phosphates. Jour. Amer. Chem. Soc. **26**: 885–913. 1904.

21. Cannon, William Austin. Physiological features of roots, with especial reference to the relation of roots to the aeration of the soil. Carnegie Inst. Wash. Pub. no. **368**: 1–168. 1925.

22. Channon, Harold John, and Chibnall, Albert Charles. The ether-soluble substances of cabbage leaf cytoplasm. V. The isolation of n-nonacosane and di-n-tetradecyl ketone. Biochem. Jour. **23**: 168–175. 1929.

23. Child, C. M. Axial susceptibility gradients in algae. Bot. Gaz. **62**: 89–114. 1916.

24. Clements, Frederick E. Aeration and air content. The rôle of oxygen in root activity. Carnegie Inst. Wash. Pub. no. **315**: 1–183. 1921.

25. Clevenger, Clinton B. Hydrogen-ion concentration of plant juices. II. Factors affecting the acidity or hydrogen-ion concentration of plant juices. Soil Sci. **8**: 227–242. 1919.

26. Comber, Norman M. The availability of mineral plant food. A modification of the present hypothesis. Jour. Agr. Sci. **12**: 363–369. 1922.

27. Crocker, William. Private communication. 1929.

28. Czapek, F. Zur Lehre von den Wurzelausscheidungen. Jahrb. wiss. Bot. **29**: 321–390. 1896.

29. ——————. Biochemie der Pflanzen, vol. 2, pp. 521–531. Jena. 1920.

30. DAVIS, A. R., HOAGLAND, D. R., and LIPMAN, C. B. The feeding power of plants. Science (n. s.) **57**: 299–301. 1923.

31. DEHÉRAIN, P. P., and DE MOUSSEY, E. Sur l'oxydation de la matière organique du sol. Ann. Agron. **22**: 305–337. 1896.

32. DENSCH, A. Versuche zur Bestimmung des Nahrstoffbedürfnisses der Böden durch Gefässversuche nach MITSCHERLICH nebst einigen Bemerkungen zur Methode NEUBAUER. Zeitschr. Pflanzenernähr. u. Düngung. **4B**: 321–339. 1925.

33. DETMER, W. Das pflanzenphysiologische Praktikum, p. 227. Jena. 1895.

34. DOMONTOVITSCH, M., and SCHESTAKOW, A. Beiträge zur Frage über die Löslichmachung von Rohphosphat durch die Wurzeln der Kulturpflanzen. Zeitschr. Pflanzenernähr. u. Düngung, **11A**: 108–112. 1929.

35. DONNAN, F. G. Theorie der Membrangleichgewichte und Membranpotentiale bei Vorhandensein von nicht Dialysierenden Electrolyten. Ein Beitrag zur physikalisch-chemischen Physiologie. Zeitschr. Elektrochem. **17**: 572–581. 1911.

36. ——————, and HARRIS, A. B. The osmotic pressure and conductivity of aqueous solutions of Congo-red and reversible membrane equilibria. Jour. Chem. Soc. (London) **99**: 1554–1577. 1911.

37. DUSTMAN, ROBERT B. Inherent factors related to absorption of mineral elements by plants. Bot. Gaz. **79**: 233–264. 1925.

38. DYER, BERNARD. On the analytical determination of probably "available" mineral plant food in soils. Jour. Chem. Soc. (London) **65**: 115–167. 1894.

39. ——————. A chemical study of the phosphoric acid and potash contents of the wheat soils of Broadbalk Field, Rothamsted. Phil. Trans. Roy. Soc. (London) B**194**: 235–290. 1901.

40. EBERMAYER, E. Mitteilungen über den Kohlensäuregehalt den Waldluft und des Waldbodens im Vergleich zu einer nicht bewaldeten Fläche. Forsch. auf dem Gebiete der Agr. Physik. **1**: 158–161. 1878.

41. ——————. Ueber den Kohlensäuregehalt eines bewaldeten und nicht bewaldeten Bodens. Landw. Versuchs-Sta. **23**: 64–66. 1879.

42. FONDER, JOHN F. Variations in potassium content of alfalfa due to stage of growth and soil type and the relationship of potassium and calcium in plants grown upon different soil types. Jour. Amer. Soc. Agron. **21**: 732–750. 1929.

43. FREAR, WILLIAM, and ERB, E. S. Condition of fertilizer potash residues in Hagerstown silty loam soil. Jour. Agr. Res. **15**: 59–81. 1918.

44. FRED, E. B., and HAAS, A. R. C. The etching of marble by roots in the presence and absence of bacteria. Jour. Gen. Physiol. **1**: 631–638. 1919.

45. FREE, E. E. The effect of aeration on the growth of buckwheat in water cultures. Johns Hopkins Univ. Circ. 198. 1917.

46. GIRARD, PIERRE. Rôle de l'electrisation de contact dans la perméabilité des membranes aux électrolytes. Compt. Rend. Acad. Sci. (Paris) **148**: 1047–1050. 1909.

47. —————, MESTREZAT, W., and LI-SHOU-HOUA. Schème physique de la perméabilité sélective des cellules vivantes aux différents ions. Compt. Rend. Acad. Sci. (Paris) **175**: 183–185. 1922.

48. —————, and MORAX, VICTOR. Échanges liquides par osmose électrique à travers des tissus vivants. Compt. Rend. Acad. Sci. (Paris) **170**: 821–823. 1920.

49. GROH, DR. Über Reaktionen der Wurzelsäfte einzelner Pflanzen und die Beeinflussung der Reaktion verschiedener Nährsalze durch die Pflanzen. Landw. Jahrb. **63**: 483–500. 1926.

50. GUSTAFSON, FELIX G. Total acidity compared with actual acidity of plant juices. Amer. Jour. Bot. **11**: 365–369. 1924.

51. —————. Diurnal changes in acidity of *Bryophyllum calycinum*. Jour. Gen. Physiol. **7**: 719–728. 1925.

52. HAAS, A. R. C. The excretion of acids by roots. Proc. Nat. Acad. Sci. **2**: 561–566. 1916.

53. —————. Studies on the reaction of plant juices. Soil Sci. **9**: 341–369. 1920.

54. —————. Private communication. 1930.

55. HALL, A. D. The solvent action of roots upon the soil particles. Sci. Prog. **1**: 51–57. 1906.

56. —————, BRENCHLEY, W. F., and UNDERWOOD, L. M. The soil solution and the mineral constituents of the soil. Phil. Trans. Roy. Soc. (London) B**204**: 179–200. 1913.

57. —————, and MILLER, N. H. J. The effect of plant growth and of manures upon the retention of bases by the soil. Proc. Roy. Soc. (London) B**77**: 1–32. 1905.

58. HANSTEEN-CRANNER, B. Beiträge zur Biochemie und Physiologie der Zellwand und der plasmatischen Grenzschichten. Ber. deutsch. bot. Ges. **37**: 380–391. 1919.

59. HASSE, P., and KIRCHMEYER, F. Die Bedeutung der Bodenatmung für die CO_2-Ernährung der Pflanzen. Zeitschr. Pflanzenernähr. u. Düngung. **10A**: 257–299. 1928.

60. HEADDEN, WILLIAM P. Effects of clover and alfalfa in rotation. I. The carbon dioxid in the soil atmosphere and its action on the

feldspar particles in the soil. Colorado Agr. Exp. Sta. Bull. 319. 1927.

61. HEMPEL, JENNY. Buffer processes in the metabolism of succulent plants. Compt. Rend. Lab. Carlsberg **13**: 1–129. 1917.

62. HESSE, H. Morphologie und Biologie der Wurzelhaare. Inaugural Dissertation. Jena. 1903.

63. HILTNER, L. Über neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderer Berücksichtigung der Gründüngung und Brache. Arb. deut. landw. Ges. **98**: 59–78. 1904.

64. HOAGLAND, D. R. The effect of the hydrogen and hydroxyl ion concentration on the growth of barley seedlings. Soil Sci. **3**: 547–560. 1917.

65. ——————. The relation of the plant to the reaction of the nutrient solution. Science (n. s.) **48**: 422–425. 1918.

66. ——————. Relation of nutrient solution to composition and reaction of cell sap of barley. Bot. Gaz. **68**: 297–304. 1919.

67. ——————. Physiological aspects of soil solution investigations. Hilgardia **1**: 227–257. 1925.

68. ——————. Private communication. 1930.

69. ——————, and DAVIS, A. R. Suggestions concerning the absorption of ions by plants. New Phyt. **24**: 99–111. 1925.

70. ——————, and SHARP, L. T. Relation of carbon dioxid to soil reaction as measured by the hydrogen electrode. Jour. Agr. Res. **12**: 139–148. 1918.

71. HÖBER, RUDOLF. Physikalische Chemie der Zelle und der Gewebe, 5th ed., pp. 587–692. Leipzig. 1924.

72. HUTCHINSON, C. M. Report of the Imperial Agricultural Bacteriologist. Rep. Agr. Res. Inst. and Coll., Pusa, India, pp. 78–83. 1911–1912.

73. JACOBS, M. H. To what extent are the physiological effects of carbon dioxide due to hydrogen ions? Amer. Jour. Physiol. **51**: 321–331. 1920.

74. ——————. The influence of ammonium salts on cell reaction. Jour. Gen. Physiol. **5**: 181–188. 1922.

75. JOHNSON, H. V. The absorption of ions by living and dead roots. Amer. Jour. Bot. **2**: 250–254. 1915.

76. JOST, LUDWIG. Lectures on Plant Physiology. Oxford. 1907.

77. KAPPEN, H. Untersuchungen an Wurzelsäften. Landw. Versuchs-Sta. **91**: 1–40. 1918.

78. KELLEY, W. P., and CUMMINS, A. B. Composition of normal and mottled citrus leaves. Jour. Agr. Res. **20**: 161–191. 1920.

79. KNOP, W. Untersuchungen über die Aufnahme der Mineralsalze durch das Pflanzengewebe. Landw. Versuchs-Sta. **6**: 81–107. 1864.

80. ——————. Chemische Untersuchungen über die Ernährung der Pflanze. Ann. Chem. Pharm. **129**: 287–333. 1864.

81. KNY, L. Über den Ort der Nährstoff-aufnahme durch die Wurzel. Ber. deut. bot. Ges. **16**: (60)–(64). 1898.

82. KÖNIG, J. Beziehungen zwischen den Eigenschaften des Bodens und der Nährstoffaufnahme durch die Pflanzen. Landw. Versuchs-Sta. **66**: 401–461. 1907.

83. KÖNIG, J., and HASENBÄUMER, J. Die Bedeutung neuer Boden-forschungen für die Landwirtschaft. Landw. Jahrb. **55**: 185–252. 1920.

84. KOSSOWITSCH, P. Über die Rolle der Pflanzen bei der Lösung der Nährstoffe des Bodens, die sich im letzteren im ungelösten Zustande befinden. Russ. Jour. Expt. Landw. **3**: 165–169. (German resumé.) 1902.

85. ——————. Die quantitative Bestimmung der Kohlensäure die von Pflanzenwurzeln während ihrer Entwickelung ausgeschieden wird. Russ. Jour. Expt. Landw. **5**: 493–500. (German resumé.) 1904.

86. ——————. Vergleichende Bestimmungen der Kohlensäure Quantitaten die von Wurzeln von Senf, Gerste, und Flache abge-schieden werden. Russ. Jour. Expt. Landw. **7**: 251–252. (German resumé.) 1906.

87. KOSTYCHEV, S. Plant Respiration. Philadelphia. 1927.

88. ——————, and BERG, V. Sur les formes des combinaisons de calcium dans les tissus des végétaux. Compt. Rend. Acad. Sci. (U. R. S. S.), ser. A: 1–6. 1929.

89. KUNZE, GUSTAV. Über Säureausscheidung bei Wurzeln und Pilz-hyphen und ihre Bedeutung. Jahrb. wiss. Bot. **42**: 357–393. 1906.

90. LAGATU, H. Sur le rôle respectif des trois bases: potasse, chaux, magnésie, dans les plantes cultivées. Compt. Rend. Acad. Sci. (Paris) **172**: 129–131. 1921.

91. ——————, and MAUME, L. Évolution remarquablement régulière de certains rapports physiologiques (chaux, magnésie, potasse) dans les feuilles de la vigne bien alimentée. Compt. Rend. Acad. Sci. (Paris) **179**: 782–785. 1924.

92. LAW, E. Beiträge zur Kenntniss der Zusammensetzung der in Acker-boden befindlichen Luft. Inaugural Dissertation. Rostock. 1906.

93. LEATHER, J. W. Soil gases. Agr. Res. Inst., Pusa, India, Mem. Dept. of Agr., Chem. Ser. **4**: 85–134. 1915.

94. LECHARTIER, G., and BELLAMY, F. De la fermentation des fruits. Compt. Rend. Acad. Sci. (Paris) **69**: 466–469. 1869.

95. LE CLERC, J. A., and BREAZEALE, J. F. Plant food removed from growing plants by rain or dew. U. S. Dept. Agr. Yearbook, pp. 389–402. 1908.

96. LEMMERMANN, OTTO. Untersuchungen über einige Ernährungsunterschiede der Leguminosen und Gramineen und ihre wahrscheinliche Ursache. Landw. Versuchs-Sta. **67**: 207–251. 1907.

97. LIEBIG, JUSTUS VON. Ueber einige Eigenschaften der Ackerkrume. Ann. Chem. Pharm. **105**: 109–144. 1858. (See p. 139).

98. LIPMAN, J. G., BLAIR, A. W., and PRINCE, A. L. The effect of lime and fertilizer on the potash content of soils and crops. International Rev. Sci. and Practice of Agr. **4**: 546–553. 1926.

99. LISSE, M. W. Private communication. 1930.

100. LOEB, JACQUES. Sodium chloride and selective diffusion in living organisms. Jour. Gen. Physiol. **5**: 231–254. 1922.

101. DE LUCA, M. S. Recherches chemiques tendant a démontrer la production de l'alcool dans les feuilles, les fleurs et les fruits de certaines plantes. Ann. Sci. Nat. Ser. 6, **6**: 286–302. 1878.

102. LUNDEGÅRDH, HENRIK. Carbon dioxide evolution of soil and crop growth. Soil Sci. **23**: 417–453. 1927.

103. ——————. Kulturväxternas kolhydratbildning, dess förhallande till avkastningen och beroende av klimatet och markbeskaffenheten. Meddelande no. 331 fran Centralanstalten för försöksväsendet pa jordbruksomradet. Avdelningen för lantbruksbotanik no. 43. 1928.

104. LYON, CHARLES J. Private communication. 1929.

105. LYON, T. L., and WILSON, J. K. Liberation of organic matter by roots of growing plants. Cornell Agr. Exp. Sta. Mem. **40**: 7–44. 1921.

106. McCOOL, M. M., and WELDON, M. D. The effect of soil type and fertilization on the composition of the expressed sap of plants. Jour. Amer. Soc. Agron. **20**: 778–792. 1928.

107. MARAIS, JACOBUS STEPHANUS. The comparative agricultural value of insoluble mineral phosphates of aluminum, iron and calcium. Soil Sci. **13**: 355–409. 1922.

108. MARTIN, THOMAS LYSONS. Decomposition of green manures at different stages of growth. Cornell Agr. Exp. Sta. Bull. 406. 139–169. 1921. And the references cited on pp. 157–158 of this bulletin.

109. Mazé, P.   Recherches sur la physiologie végétale.   I. Nutrition minérale des végétaux.   Absorption et excrétion des éléments minéraux par les racines et par les feuilles.   Excrétion des substances organiques.   Ann. Inst. Pasteur **25**: 705–738.   1911.

110. Melin, E.   Untersuchungen über die Bedeutung der Baummykorhiza eine ökologisch-physiologische Studie.   Jena.   1925.

111. Metzger, W. H.   The effect of growing plants on solubility of soil nutrients.   Soil Sci. **25**: 273–280.   1928.

112. ————————.   Private communication.   1929.

113. Meulen, P. A. Van der, and Rieman, 3rd, Wm.   Monomolecular films of sodium ricinoleate in emulsions.   Jour. Amer. Chem. Soc. **46**: 876–880.   1924.

114. Mevius, Walter.   Kalzium-Ion und Wurzelwachstum.   Jahrb. wiss. Bot. **66**: 183–253.   1927.

115. Miller, Edwin C.   The root systems of agricultural plants.   Jour. Amer. Soc. Agron. **8**: 129–154.   1916.

116. Minina, E. G.   On the acidity of root secretions.   Bull. Inst. Recherches Biol. Univ. de Perm. **5**: 238–258.   1927.   (Russian with English summary.)

117. Mitscherlich, E. A.   Eine chemische Bodenanalyse für pflanzenphysiologische Forschungen.   Landw. Jahrb. **36**: 309–369.   1907.

118. ————————.   Ein Beitrag zur Kohlensäuredüngung.   Landw. Jahrb. **39**: 157–166.   1910.

119. ————————.   Eine Bestimmung des Düngerbedürfnisses des Bodens.   Zweite Auflage.   Berlin.   1925.

120. ————————.   Private communication.   1929.

121. ————————, and Celichowski, K.   Ein Beitrag zur Erforschung der Ausnutzung des im Minimum vorhandenen Nährstoffes durch die Pflanze.   Landw. Jahrb. **39**: 133–156.   1910.

122. Molisch, H.   Über Wurzelausscheidungen und deren Einwirkung auf organische Substanzen.   Sitzungsber. d. Akad. Wiss. (Vienna), **96**: 84–109.   1888.

123. Möller, Joseph.   Über die freie Kohlensäure im Boden.   Forsch. auf dem Gebiete der Agrik. Physik. **1**: 158–161.   1879.

124. Neller, J. R.   The influence of growing plants upon oxidation processes in the soil.   Soil Sci. **13**: 139–159.   1922.

125. Němec, Antonin.   Sur la concntration en ions hydrogène dans le tissu des graines.   Compt. Rend. Acad. Sci. (Paris) **180**: 1776–1778.   1925.

126. Newton, J. D.   A comparison of the absorption of inorganic elements, and of the buffer systems of legumes and non-legumes, and its bearing upon existing theories.   Soil Sci. **15**: 181–204.   1923.

127. —————————. Measurements of carbon dioxide evolved from the roots of various crop plants. Sci. Agr. **4**: 268–274. 1924.

128. —————————. The relation of the salt concentration of the culture solution to transpiration and root respiration. Sci. Agr. **5**: 318–320. 1925.

129. —————————. The selective absorption of inorganic elements by various crop plants. Soil Sci. **26**: 85–91. 1928.

130. —————————. Private communication. 1929.

131. NOBBE, FRIEDERICH. Ueber die feinere Verästelung der Pflanzenwurzel. Landw. Versuchs-Sta. **4**: 212–224. 1862.

132. OAKLEY, HENRY BOWEN. The origin of the charge on colloidal particles. Jour. Phys. Chem. **30**: 902–916. 1926.

133. OSTERHOUT, W. J. V. Some chemical relations of plant and soil. Science (n.s.) **36**: 571–576. 1912.

134. —————————. Some aspects of selective absorption. Jour. Gen. Physiol. **5**: 225–230. 1922.

135. —————————. Is living protoplasm permeable to ions? Jour. Gen. Physiol. **8**: 131–146. 1925.

136. —————————. Some aspects of bioelectrical phenomena. Jour. Gen. Physiol. **11**: 83–99. 1927.

137. —————————, and DORCAS, M. J. The penetration of CO_2 into living protoplasm. Jour. Gen. Physiol. **9**: 255–267. 1925.

138. PANTANELLI, E. Über Ionenaufnahme. Jahrb. wiss. Bot. **56**: 689–733. 1915.

139. PARKER, F. W. Carbon dioxide production of plant roots as a factor in the feeding power of plants. Soil Sci. **17**: 229–247. 1924.

140. —————————. The carbon dioxide content of the soil air as a factor in the absorption of inorganic elements by plants. Soil Sci. **20**: 39–44. 1925.

141. —————————. Soil phosphorus studies. III. Plant growth and the absorption of phosphorus from culture solutions of different phosphate concentrations. Soil Sci. **24**: 129–146. 1927.

142. —————————, and TRUOG, E. The relation between the calcium and the nitrogen content of plants and the function of calcium. Soil Sci. **10**: 49–56. 1920.

143. PAVLINOVA, E. Physiological significance of guttation. Bull. Inst. Recherches Biol. Univ. de Perm. **4**: 471–478. 1926. (Russian with English summary.)

144. PEPIN, J. AUGUSTE. The rôle of calcium in Dunkirk and Volusia soils. Sci. Agr. **5**: 139–154. 1925.

145. PFEIFFER, TH., and BLANCK, E. Die Säureausscheidung der Wurzeln und die Löslichkeit der Bodennährstoffe in Kohlensäurehaltigem Wasser. Landw. Versuchs-Sta. **77**: 217–268. 1912.

146. ————, ————, SIMMERMACHER, W., and RATHMANN, W. Pflanzenanalyse und Bodenanalyse zur Bestimmung des Nährstoffgehaltes der Ackerböden. Landw. Versuchs-Sta. **86**: 339–391. 1915.

147. ————, SIMMERMACHER, W., and SPANGENBERG, M. Die Löslichkeit verschiedener Phosphate und deren Ausnutzung durch Hafer und Buchweizen. Landw. Versuchs-Sta. **89**: 203–230. 1917.

148. PIERRE, W. H., and PARKER, F. W. Soil phosphorus studies. II. The concentration of organic and inorganic phosphorus in the soil solution and soil extracts and the availability of the organic phosphorus to plants. Soil Sci. **24**: 119–128. 1927.

149. PLUMMER, J. K. Some effects of oxygen and carbon dioxide on nitrification and ammonification in soils. Cornell Agr. Exp. Sta. Bull. **384**. 301–330. 1916.

150. POTTER, R. S., and SNYDER, R. S. Carbon dioxid production in soils and carbon and nitrogen changes in soils variously treated. Iowa Agr. Exp. Sta. Res. Bull. 39. 253–309. 1916.

151. PRIANISHNIKOV, D. Über die Ausnutzung der Phosphorsäure der schwerslöslichen Phosphate durch höhere Pflanzen. Ber. deut. bot. Ges. **18**: 411–416. 1900.

152. ————. Zur Frage über den relativen Wert von verschiedenen Phosphaten. Landw. Versuchs-Sta. **56**: 107–140. 1902.

153. ————. Zur Frage über die Wurzelausscheidungen. Ber. deut. bot. Ges. **22**: 184–191. 1904.

154. ————. Zur Frage über den relativen Wert verschiedener Phosphate. (II. Mitteilung.) Landw. Versuchs-Sta. 65: 23–54. 1907.

155. ————. Über den Einfluss von kohlensäurem Kalk auf die Wirkung von verschiedenen Phosphaten. Landw. Versuchs-Sta. **75**: 357–376. 1911.

156. PURIEWITSCH, K. Die Bildung und Zersetzung der organischen Säuren bei den höheren Pflanzen. Kiev. 1893.

157. REED, H. S., and HAAS, A. R. C. Nutrient and toxic effects of certain ions on citrus and walnut trees with especial reference to the concentration and pH of the medium. Univ. California Pub. Tech. Paper no. 17: 1–75. 1924.

158. ——————, and ——————. Some relations between the growth and composition of young orange trees and the concentration of the nutrient solution employed. Jour. Agr. Res. **28**: 277–284. 1924.

159. RIEMAN, 3rd, WM., and VAN DER MEULEN, P. A. Monomolecular soap films in emulsions. Jour. Amer. Chem. Soc. **47**: 2507–2514. 1925.

160. RUDOLFS, WILLEM. Effect of seeds upon hydrogen-ion concentration of solutions. Bot. Gaz. **74**: 215–220. 1922.

161. ——————. Effect of seeds upon hydrogen-ion concentration equilibrium in solution. Jour. Agr. Res. **30**: 1021–1026. 1925.

162. RUSSELL, E. J. Soil conditions and plant growth. New York. 1927.

163. ——————, and APPLEYARD, A. The atmosphere of the soil: Its composition and the causes of variation. Jour. Agr. Sci. **7**: 1–48. 1915.

164. ——————, and ——————. The influence of soil conditions on the decomposition of organic matter in the soil. Jour. Agr. Sci. **8**: 385–417. 1917.

165. SACHS, J. Physiologische Mittheilungen verschiedenen Inhaltes. II. Auflösung des Marmors durch Maiswurzeln. Bot. Zeit. **18**: 117–118. 1860.

166. SACKETT, WALTER G., KEZER, A., FERGUSON, IDA W., and WARD, JUSTUS C. The effect of green manures and crop residues on soil reaction. Colorado Agr. Exp. Sta. Bull. 324: 1–31. 1928.

167. DE SAUSSURE, TH. Chemische Untersuchungen über die Vegetation. Ostwalds Klassiker der exakten Wissenschaften nos. 15 und 16. Leipzig. 1890.

168. SCHLOESSING, TH. Contribution à l'étude de l'alimentation des plantes en phosphore. Compt. Rend. Acad. Sci. (Paris) **134**: 53–55. 1902.

169. SCHREINER, OSWALD, and SULLIVAN, M. X. Studies in soil oxidation. U. S. Dept. Agr. Bur. Soils Bull. 73. 1910.

170. SHARP, L. T., and HOAGLAND, D. R. Acidity and adsorption in soils as measured by the hydrogen electrode. Jour. Agr. Res. **7**: 123–145. 1916.

171. SHEDD, O. M. Effect of certain calcium compounds and other substances on the yield and calcium content of some crops. Soil Sci. **14**: 233–246. 1922.

172. SHULL, C. A. Private communication. 1929.

173. SIMMS, HENRY S. Chemical antagonism of ions. I. Effects of Na–Mg and K–Mg mixtures on the activity of oxalic diion: Jour. Gen. Physiol. **12**: 241–258. 1928.

174. ——————. Chemical antagonism of ions. III. Effects of salt mixtures on gelatin activity. Jour. Gen. Physiol. **12**: 511–528. 1929.

175. SKEEN, JOHN R. Some reactions of seedlings to weak concentrations of hydrochloric acid and calcium. Soil Sci. **26**: 471–478. 1928.

176. SPOEHR, H. A., and McGEE, J. M. Studies in plant respiration and photosynthesis. Carnegie Inst. Wash. Pub. no. 325: 1–98. 1923.

177. STARKEY, ROBERT L. Some influences of the development of higher plants upon the microorganisms in the soil. I. Historical and introductory. Soil Sci. **27**: 319–334. 1929.

178. ——————. Some influences of the development of higher plants upon the microorganisms in the soil. III. Influence of the stage of plant growth upon some activities of the organisms. Soil Sci. **27**: 434–444. 1929.

179. STILES, WALTER, and JÖRGENSEN, INGVAR. Observations on the influence of aeration of the nutrient solution in water culture experiments, with some remarks on the water culture method. New Phyt. **16**: 181–197. 1917.

180. STOKLASA, JULIUS. Sur la respiration des racines. Compt. Rend. Acad. Sci. (Paris) **175**: 995–997. 1922.

181. ——————. Über die Resorption der Ionen durch das Wurzelsystem der Pflanzen aus dem Boden. Ber. deut. bot. Ges. **42**: 183–191. 1924.

182. ——————, and ERNEST, ADOLF. Ueber den Ursprung, die Menge und die Bedeutung des Kohlendioxyds im Boden. Centr. für Bakt. II Abt. **14**: 723–736. 1905.

183. ——————, and ——————. Beiträge zur Lösung der Frage der chemischen Natur des Wurzelsekretes. Jahrb. wiss. Bot. **46**: 55–102. 1908.

184. STORCK, ALFRED, and RIPPEL, AUGUST. Bestimmung der Stickstoffbindung bei Leguminosen vermittels des Stickstoff-Basenverhältnisses. Zeitschr. Pflanzenernähr. u. Düngung. **13**A: 158–159. 1929.

185. THERON, J. J. Influence of reaction on interrelations between the plant and its culture medium. II. Possible mechanism of the plant's influence on the reaction of the culture solution. Univ. California Pub. Agr. Sci. **4**: 437–444. 1924.

186. THOMAS, WALTER. Soil investigations. Unpublished. 1920–1921.

187. ——————. Studies of certain phases of the interrelationship between soil and plant. I. Availability of mineral plant nutrients in relation to the degree of dispersion. Soil Sci. **27**: 249–270. 1929.

188. ——————. Balanced fertilizers and Liebig's law of the minimum. Science (n. s.) **70**: 382–384. 1929.

189. ——————. The effect of colloidal silica on the absorption of phosphoric acid by plants. Science n. s. **71**: 422–423. 1930.

190. ——————, and ANTHONY, R. D. Eliminating some of the variables in apple fertilizer experiments. Proc. Amer. Soc. Hort. Sci., pp. 81–87. 1926.

191. TRUOG, E. Factors influencing the availability of rock phosphate. Wisconsin Agr. Exp. Sta. Res. Bull. **20**: 17–51. 1912.

192. ——————. The utilization of phosphates by agricultural crops, including a new theory regarding the feeding power of plants. Wisconsin Agr. Exp. Sta. Res. Bull. **41**: 1–50. 1916.

193. ——————. Soil acidity. I. Its relation to the growth of plants. Soil Sci. **5**: 169–195. 1918.

194. ——————. The feeding power of plants. Science (n. s.) **56**: 294–298. 1922.

195. ——————, and MEACHAM, M. R. Soil acidity. II. Its relation to the acidity of the plant juice. Soil Sci. **7**: 469–474. 1919.

196. TSCHIRIKOW, TH. W. Zur Frage über die lösende Wirkung der Wurzeln. Russ. Jour. Expt. Landw. **15**: 54–65. 1914.

197. ——————. Einige Angaben über die Frage der Löslichkeitsfähigkeit der Wurzeln höherer Pflanzen. Parashnikow's Lab. Erg. Veg. und Laboratoriumsversuche **11**: 1–25. 1918. (Russisch mit Französischen Resumé.)

198. TURPIN, H. W. The carbon dioxide of the soil air. Cornell Agr. Exp. Sta. Mem. **32**: 319–362. 1920.

199. VAN SLYKE, DONALD D. Studies of acidosis. II. A method for the determination of carbon dioxide and carbonates in solution. Jour. Biol. Chem. **30**: 347–368. 1917.

200. WALKER, JAMES, and CORMACK, WILLIAM. The dissociation constants of very weak acids. Jour. Chem. Soc. (London) **77**: 5–21. 1900.

201. WARMING, E. Ecology of plants. Oxford. 1925.

202. WEAVER, J. E., and HIMMEL, W. J. Relation between the development of root system and shoot under long- and short-day illumination. Plant Physiol. **4**: 435–457. 1929.

203. WEISER, HARRY B. Colloid Symposium Monograph, Vol. **4**: 354–373. New York. 1926.

204. WERTHEIMER, E. Weitere Studien über die Permeabilität lebender Membranen. VI. Über die Permeabilität von Säuren und Basen. Einfluss der Temperatur auf die Permeabilität. Arch. Ges. Physiol. (Pflüger's) 203: 542–557. 1924.

205. ——————. Weitere Untersuchungen an der lebenden Froschhautmembran. VII. Mitteilung. Arch. Ges. Physiol. (Pflüger's) 206: 162–171. 1924.

206. WHITE, J. W. Soil organic matter and manurial treatments. Jour. Amer. Soc. Agron. 19: 389–396. 1927.

207. WILFARTH, H., RÖMER, H., and WIMMER, G. Über die Nährsstoffaufnahme der Pflanzen in verschiedenen Zeiten ihres Wachstums. Landw. Versuchs-Sta. 63: 1–70. 1906.

208. WILSON, J. K. Device for growing large plants in sterile media. Phytopath. 10: 425–429. 1920.

209. WOLFF, EMIL. Bericht über die in den Jahren 1866 und 1867 ausgeführten Vegetationsversuche in wässriger Lösung der Nährstoffe. Landw. Versuchs-Sta. 10: 349–379. 1868.

210. ——————. Aschen-analysen von landwirtschaftlichen Producten. Berlin. 1871.

211. WOLLNY, E. Untersuchungen über den Einfluss der Pflanzendecke und der Beschattung auf den Kohlensäuregehalt der Bodenluft. Forsch. auf dem Geibeite der Agr. Physik. 3: 1–15. 1880.

212. ——————. Untersuchungen über den Kohlensäuregehalt der Bodenluft. Landw. Versuchs-Sta. 25: 373–391. 1880.

213. ——————. Die Zersetzung der organischen Stoffe und die Humus Bildungen. 1897.

214. WRIGHT, NORMAN CHARLES. Membrane equilibria and selective absorption. Biochem. Jour. 23: 352–357. 1929.

ELECTRIC CORRELATION POTENTIALS IN THE LEAF OF *BRYOPHYLLUM*

E. J. Lund and M. Bush

(WITH THREE PLATES AND NINE FIGURES)

Introduction

It has long been known that some forms of living tissues have the peculiar property of producing a continuous output of electrical energy under normal conditions of growth and development. These continuously existing differences of potential have been studied more extensively in plants than in animals by early investigators. Buff (3), Kunkel (7), Elfving (5), Burdon-Sanderson (4), and Müller-Hettlingen (14). Later Mathews (13) found that differences of potential occurred in hydroid stems of *Tubularia, Pennaria,* and *Campanularia,* and that the structural polarity of these hydroids was accompanied by electrical polarity. More recently, the magnitude, direction, and distribution of maintained electric potentials have been determined in the stem of *Obelia,* Lund (8), and in onion roots, Lund and Kenyon (11). Later experiments showed a quantitative relation between cell oxidation and continuously maintained bio-electric currents in such polar tissues as *Obelia,* frog skin, and roots, Lund (9). These findings have led to the formulation of a theory which expresses the relation between organic polarity, cell oxidation, and continuously maintained differences of electric potential within the same cell or organism. In the onion root, Marsh (12) and in the Douglas fir and white fir, Lund (10), it has been demonstrated that each cell in any given length of tissue is the seat of an individual E. M. F. such that the potential difference which occurs between two points on the tissue, represents an algebraic sum of all the individual E. M. F.'s of the cells in the region over which the potential is measured.

The following experiments were carried out for the purpose of extending the observations on continuously maintained differences of electric potential to a diversity of living material, and to further test the principle of summation of E. M. F.'s.

Plants, such as *Coleus, Cosmos,* and *Bryophyllum,* were used in preliminary experiments, and all of them exhibited continuously maintained differences of potential along the main axis. *Bryophyllum* was selected for further experiments. This well-known plant furnishes especially interesting material because of its peculiar habit of producing shoots from growing points at the notches of the leaf after it is detached (plate V).

In the experiments which follow, different pairs of points on the leaf were selected for measurement of potential difference in order to determine:

(1) whether continuous electric potentials were maintained between the growing points, (2) whether each growing point developed a potential independently of other growing points, and (3) whether the growing points differed from other parts of the leaf in the development of inherent electric potential. The final problem was to determine whether or not evidence of algebraic summation of E. M. F.'s would be found in suitable preparations of the leaf.

Experimental material consisted of potted plants of *Bryophyllum* in various stages of active, normal growth.

Methods and apparatus

Potentials were measured by a Leeds and Northrup hydrogen ion potentiometer. Readings were expressed in millivolts. The limit of accuracy under the conditions of the experiments was less than one millivolt.

The arrangement of the apparatus is shown in plate VI. A moist chamber, A, was supported on a stand equipped with rack and pinion, F. In the center of the floor of the chamber was a paraffined cork on which the leaf preparation, L, rested during experiments. In the floor of the chamber were five perforations to admit the tubes from the electrode cups.

The glass tubes from the electrode cups, D and E, were joined by rubber tubing to bent glass tubes, B and C, which ended at the tip in two claw-like glass projections. The claw fitted loosely over the growing point and insured a good water contact. If one contact was to be made at a surface of a leaf preparation, then a straight tip, with a capillary opening was used instead of the bent claw. The electrode cups were supported by means of special adjustable clamps which were attached to stands, G and H, each equipped with rack and pinion. The clamps and the rack and pinions facilitated the adjustment of the contact tips. The electrode cups and tubes were filled with tap water which formed contacts with the leaf preparation. The water in the cups was kept level with that at the contact tips.

Positive and negative electrodes, P and N, respectively, dipped into the cups as represented. The electrodes were of cadmium amalgam-$CdSO_4$ type.

The leads from the electrodes passed to K which is a large paraffin block supported by a wooden block, and which contained six cups filled with mercury. The cups were connected by copper keys with sealing wax handles.

The potential difference of the electrode system was measured each time and subtracted from the potential difference found when the leaf preparation was in the circuit. It was necessary to take the measurement of the electrodes rather frequently, since the potential difference of the electrode system changed somewhat when an experiment was carried on for several

hours. The variation was 0.5 to 1.5 millivolts in two or three hours when the $CdSO_4$ electrodes were used.

After a leaf preparation was placed within the moist chamber, and the electrode tips were adjusted, a few minutes were allowed to elapse in order to permit the leaf preparation to recover from the stimulation which resulted from the cutting and the placing on the electrode contacts. Care was then taken not to touch the preparation during an experiment. Since the moist chamber, A, was lined with moist filter paper, the amount of water at the contacts remained constant. Any renewal or changing of the water at the contact "stimulated" (?) the tissue at the point of contact. This was verified by making measurements of the potential difference before renewing a contact and immediately afterward. The latter showed a much greater variation and more rapid changes in potential than those before the contact was renewed.

Experimental

In preliminary experiments, measurements of difference of potential were made on *Coleus, Cosmos,* and *Bryophyllum,* particularly between different points along the length of the stems. In order to measure the potential from the entire stem, a little strand of wet cotton was placed around the stem at the level of measurement and contact was made with the cotton. The cotton also prevented the possibility of the electrode tip coming in contact with the stem and injuring or stimulating it. The older, basal, hardened parts of the stems of *Cosmos* and *Bryophyllum* show smaller differences of potential than corresponding lengths of the newer, actively growing parts of the same stem. These observations are in agreement with the observations on roots, the *Obelia* stem, and the Douglas fir.

The special experiments which follow, were made with *Bryophyllum* leaves, or parts of leaves.

1. DIFFERENCES OF POTENTIAL BETWEEN THE PETIOLE AND SELECTED POINTS ON THE SURFACE OF THE LEAF BLADE

Experiments were performed using the entire leaf and measuring the differences of potential between the distal part of the petiole and (1) each of four selected growing points (plate VII, fig. 1), and (2) each of four selected points on the ventral side of the leaf (plate VII, fig. 2). In each case these measurements were made while the leaf was attached to the plant, and again when the leaf was detached. The petiole was wrapped loosely with a little strand of wet cotton, and rested between the glass projections of the electrode tip. The results of these and following experiments are recorded by means of graphs. In these, the abscissa represents time in minutes and the ordinate represents readings in millivolts. In figures 1, 2, 3, and 4 the
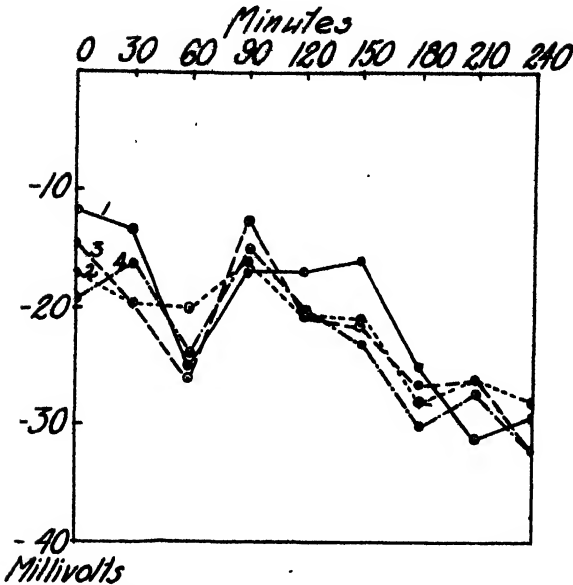
FIG. 1. Curves representing the P.D.'s between the petiole and each one of four selected growing points while the leaf was attached to the plant. Curves 1, 2, 3, 4 correspond to the P.D.'s between the points 1, 2, 3, 4, and the point on the petiole marked + in plate VII, 1.

negative signs indicate that the growing points and the selected points on the lamina are negative to the distal part of the petiole. The numbers of the graphs correspond to the numbers of the points indicated in plate VII, figures 1 and 2.

Figure 1 represents the differences of potential between the distal petiole and each of four selected growing points, while the leaf was attached. Figure 2 (a) represents the differences of potential between the same points immediately after the leaf was detached. Figure 2 (b) represents the differences of potential between the same points five days after the leaf was detached, when the growing points were beginning to sprout.

The leaf removed showed greater variations than the same leaf on the plant. It showed somewhat greater differences of potential a short time after being detached than it did five days later. The temperature of the room was constant to within two degrees during these tests.

Figure 3 represents the differences of potential between the distal part of the petiole and four selected points on the ventral side of the leaf, while the leaf was attached. Note that the potential differences are less variable than those between the growing points and the petiole. Figure 4 represents differences of potentials between the same points after the leaf was detached. In this experiment also, the detached leaf showed an increase in
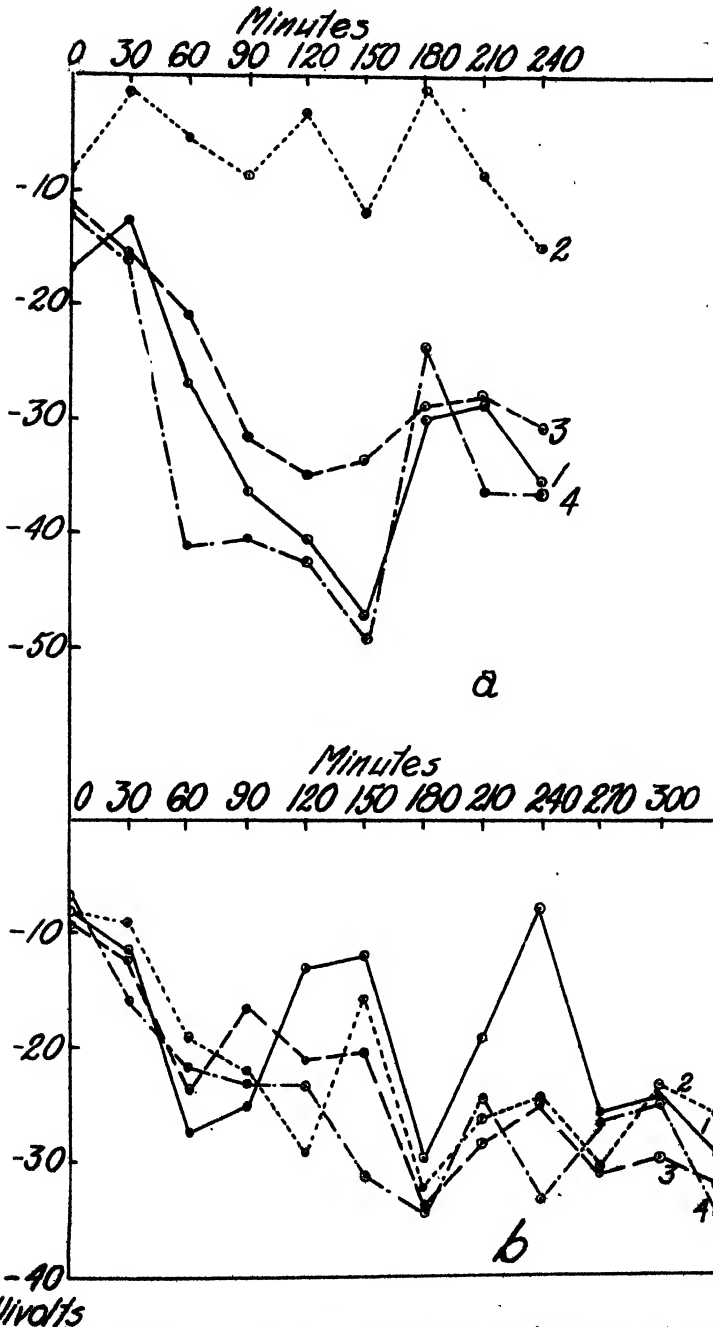
FIG. 2. a. Curves showing the P.D.'s between the same points of the leaf as those in fig. 1, immediately after the leaf was detached. b. Same as a, but taken five days after the leaf was detached. Note the difference between the different points.
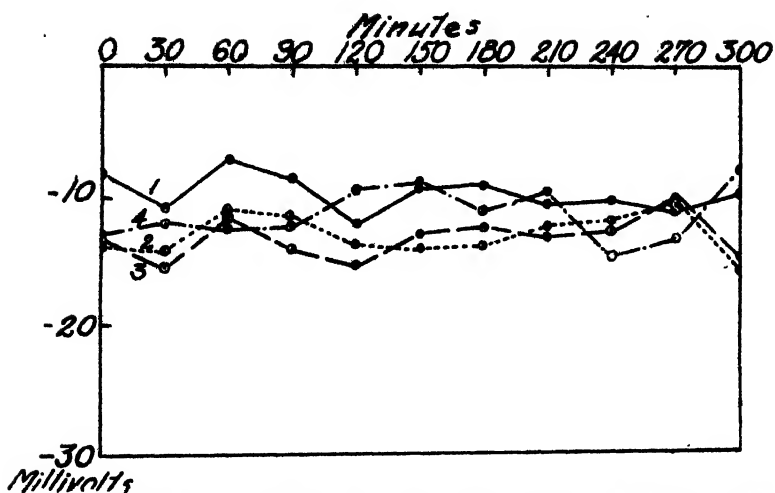
FIG. 3. Curves showing the P.D.'s between the petiole and four selected points on the
ventral side of the same leaf as that of figs. 1 and 2, while the leaf was
attached to the plant. Compare with curves of fig. 1.
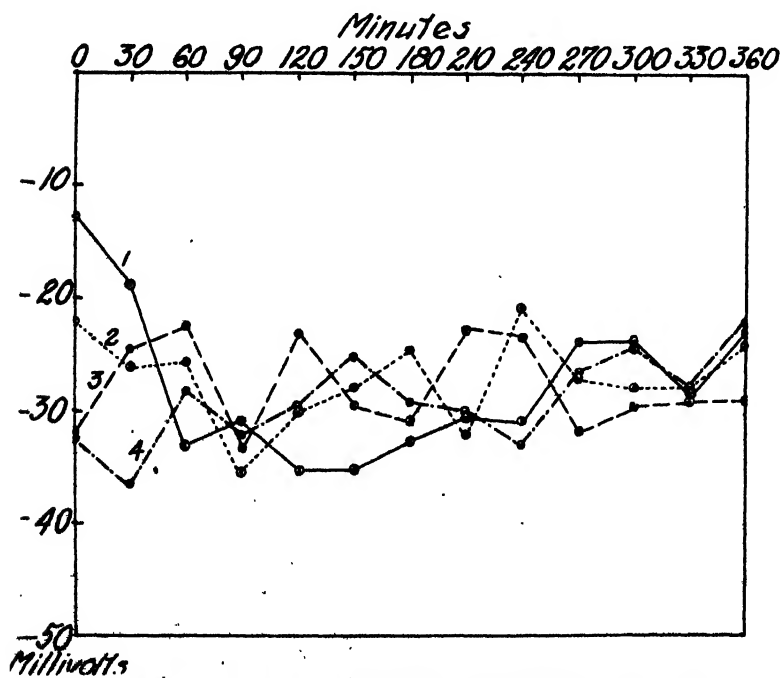
FIG. 4. Same as fig. 3, after removal of the leaf from the plant.

differences of potential and in variation, the room temperature being the same during the tests.

These experiments show the petiole to be positive to the growing points and to points on the lamina. They also show that under what may be considered constant external conditions (absence of external "stimulus") the processes which develop the maintained electric potentials, change from instant to instant. In this respect they resemble the potentials in roots. The curves show that the potential differences between different growing points and the same point on the petiole are within certain limits independent variables. The curves clearly indicate that in general local processes determine the magnitude of local potentials, or, in other words, the observed potentials are the algebraic sum of a complex system of E. M. F.'s of diverse, internal, local origins. The results amplify and confirm the observations on roots, *Obelia*, and the Douglas fir.

In previous work on electrical phenomena in plants and animal tissues it has been the practice to ignore electric potentials of the type referred to in this paper, if they were not overlooked. Often if such potentials were observed, an attempt was made to compensate for them by applying a counter electromotive force before the application of an external stimulus, the effects of which were the objects of the study. A typical example of such procedure is found in the work by FRASER (6). Here emphasis is therefore placed upon the fact that the causes of the observed variations are largely if not entirely within the tissue system itself.

2. DIFFERENCES OF POTENTIAL BETWEEN TWO GROWING POINTS ON OPPOSITE SIDES OF A LEAF AND BETWEEN TWO ADJACENT GROWING POINTS

A narrow strip of a leaf, containing two growing points from opposite sides of a leaf was prepared (plate VII, fig. 3). Figure 5, a and b, represents the result of the readings from two such preparations. As shown by the graphs, the points were alternately positive and negative in relation to each other. The magnitude of potential at a growing point varied continuously so that, sometimes, one of the growing points had a greater potential, and was, therefore, positive to the second. Then, again, the second growing point showed a higher potential and became positive to the first. Zero indicated that the potentials of the two growing point systems were equal and oppositely directed and, consequently, there was no deflection of the galvanometer.

A similar preparation was made, but instead of having the growing points connected by leaf tissue, they were connected by a water bridge (plate VII, fig. 4). The bridge consisted of a narrow piece of filter paper which prevented the water from spreading. In this preparation also, the growing

points alternated positive and negative as shown in figure 6. This experiment showed that the growing points were not dependent on each other in the development of potential, since they were not connected by leaf tissue.

A preparation consisting of a narrow strip of leaf with two adjacent growing points was made (plate VII, fig. 5) and potential differences between the two growing points were measured. The path of the circuit in this case was in the leaf tissue between the two growing points. Figure 7 represents the results of a typical experiment. The potential of the growing points in this preparation also alternated positive and negative in relation to each other.
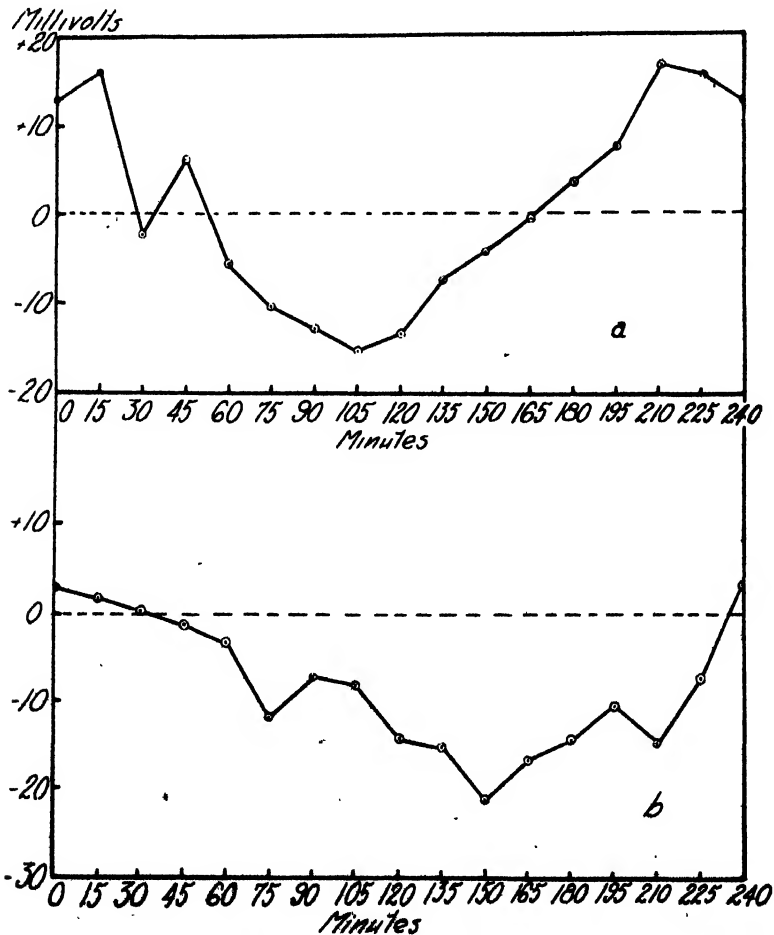
FIG. 5. *a.* and *b.* Curves showing the P.D.'s between oppositely directed growing points of two different leaf preparations as shown in plate VII, 3. Note the spontaneous reversals of orientation of total E. M. F.
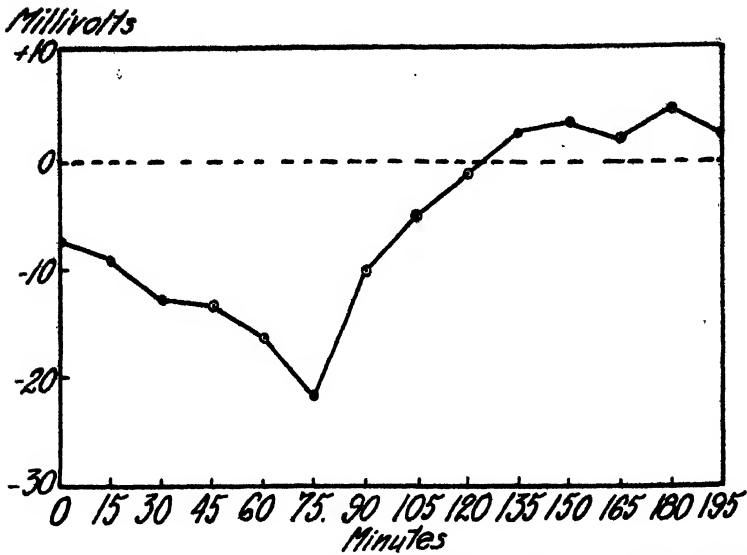
FIG. 6. Curve showing the P.D. between two opposite oriented growing points when the tissue pieces were connected as shown in plate VII, 4.
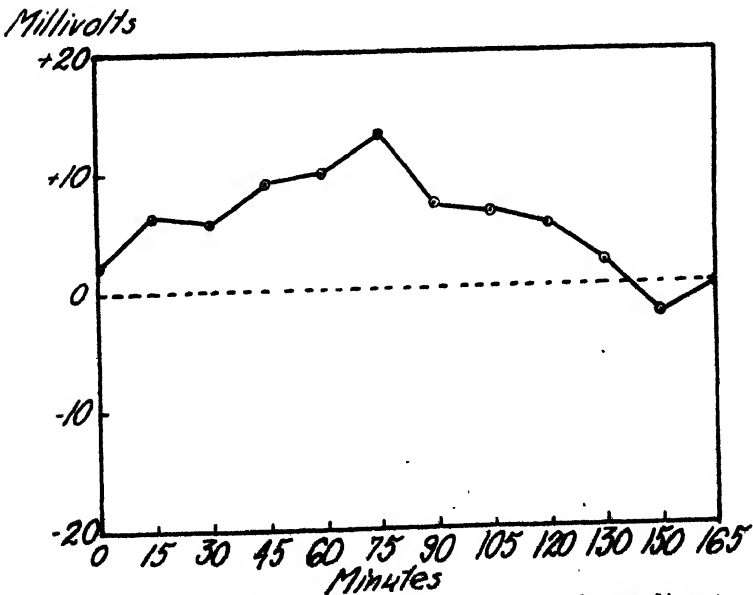
FIG. 7. Curve showing variation and reversal of orientation of two adjacent growing points as shown in plate VII, 5.

3. Differences of Electric Potential Between Growing Points and Other Points on the Leaf Surface

In order to determine the relation of potentials of the growing points to the other parts of the leaf, measurements were made, (1) between a growing point and a point on the lamina (plate VII, fig. 6) and (2) between a growing point and the tip of the adjacent lobe (plate VII, fig. 7). Figure 8
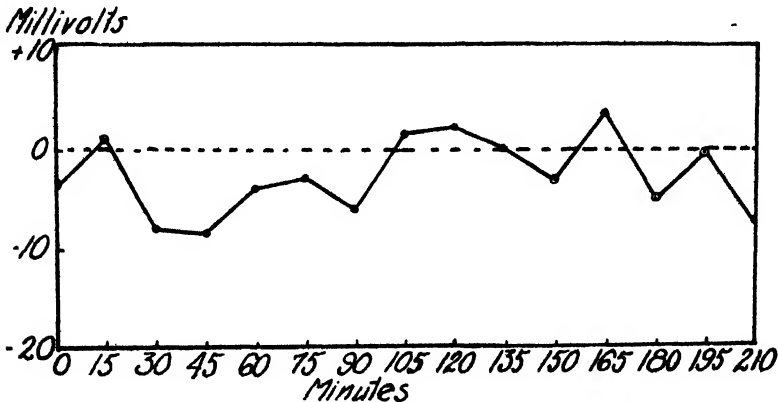
Fig. 8. Curve of P.D. between a growing point and a point on the ventral side of the leaf as shown in plate VII, 6. Note that the growing point is not always positive.

shows the results of one series of readings. The growing point was not always positive to the point on the lamina. In the second series of measurements, represented by figure 9, the growing point was without exception
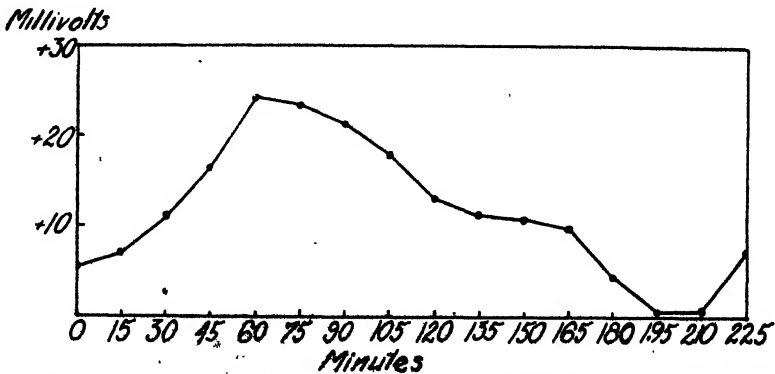
Fig. 9. Curve of P.D. between a growing point and the tip of the lobe between two adjacent growing points as shown in plate VII, 7. See table I.

positive to the tip of the adjacent lobe. Many measurements were made using this sort of preparation, four or five readings at fifteen minute inter-

vals were made on each one of these preparations, but no reading showed the growing point negative to the tip of the lobe. Results of these readings are given in table I. These results show without a single exception that the growing point develops an electric potential greater than, and independent of that produced by the surrounding tissue.

TABLE I

DIFFERENCES OF POTENTIAL IN MILLIVOLTS BETWEEN A GROWING POINT AND A POINT AT THE TIP OF AN ADJACENT LOBE. SUCCESSIVE MEASUREMENTS ON THE SAME LEAF STRIP TAKEN AT FIFTEEN MINUTE INTERVALS

PREPARATION	1*	2	3	4	5	6
	+ 1.8	+ 2.5	+ 3.3	+ 3.0	+ 5.3	+ 3.5
	+ 3.6	+ 4.8	+ 9.3	+ 6.5	+ 7.3	+ 8.2
	+11.2	+ 9.5	+11.0	+ 8.2	+10.6	+ 8.0
	+12.8	+10.2	+12.3	+10.2	+19.0	+ 6.5
Average	+ 6.8	+ 6.7	+ 8.9	+ 6.9	+10.5	+ 6.5

PREPARATION	7	8	9	10	11	12
	+ 3.0	+ 2.1	+ 9.5	+ 9.4	+ 7.0	+ 3.4
	+ 7.2	+ 8.1	+ 1.5	+ 4.8	+ 7.2	+ 8.4
	+ 6.2	+ 7.6	+ 5.5	+ 5.6	+ 8.5	+ 9.6
	+ 9.5	+ 9.4	+ 6.0	+ 4.8	+10.5	+16.1
Average	+ 6.4	+ 6.8	+ 5.6	+ 6.1	+ 8.3	+ 9.3

PREPARATION	13	14	15	16	17	18
	+ 4.0	+ 4.2	+ 3.0	+ 5.0	+ .8	+ 2.5
	+ 5.4	+ 7.8	+ 7.5	+ 8.5	+ 3.5	+ 4.5
	+ 8.3	+12.0	+15.0	+16.8	+ 6.5	+ 8.0
	+14.5	+20.0	+19.5	+20.0	+16.0	+19.0
Average	+ 8.0	+11.0	+11.2	+12.5	+ 6.7	+ 8.5

PREPARATION	19	20	21	22		
	+ 1.0	+ 3.0	+ 2.3	+ 5.5		
	+ 2.5	+ 5.0	+ 5.7	+ 7.7		
	+ 8.8	+12.5	+ 9.5	+ 9.5		
	+10.0	+15.7	+17.5	+18.0		
Average	+ 5.5	+ 9.0	+ 8.7	+10.1		

* The numbers at the tops of the columns indicate preparations (leaf strips).

+ The + sign indicates that the growing point is positive to the point on the lobe.

4. EVIDENCE FOR ALGEBRAIC SUMMATION OF E. M. F.'s IN A STRIP ACROSS A LEAF OF *Bryophyllum*

In the first experiment, a preparation consisting of two growing points on opposite sides of the leaf, connected by a very narrow strip of leaf tissue was used (plate VII, fig. 8). The electrode tips with glass projections were fitted loosely at A and C, and B rested between the glass projections. By trying out various combinations of positive and negative electrodes, it was found that A and C were each positive to B, and in this particular experiment, A was positive to C in all readings made. The measurements of difference of potential between A and B, B and C, and A and C are given in table II. The readings for A–B and B–C were subtracted algebraically and

TABLE II

POTENTIAL DIFFERENCES IN MILLIVOLTS BETWEEN TWO GROWING POINTS ON OPPOSITE SIDES OF A LEAF, CONNECTED BY A NARROW STRIP OF LEAF TISSUE.
(CONTACTS AS SHOWN IN PLATE III, FIGURE 8)

PD A——B + −	PD B——C − +	EXPECTED PD A——C + −	OBSERVED PD A——C + −	DIFFERENCE IN MILLIVOLTS
+ 32.7	− 48.0	− 15.3	− 10.5	4.8
+ 30.7	− 50.3	− 19.6	− 15.8	3.8
+ 27.0	− 43.7	− 16.7	− 14.0	2.7
+ 32.3	− 41.1	− 8.8	− 10.0	1.2
+ 30.3	− 37.1	− 6.8	− 10.0	3.2
+ 29.5	− 42.7	− 13.2	− 18.0	4.8

the result was compared with the actual reading for A–C. According to our limit of error, the difference between the expected differences of potential and the observed difference of potential should not have been greater than two millivolts. Since the results showed an average difference of 3.1 millivolts we may conclude that in this type of preparation, an exact summation did not occur. The differences were probably due to the fact that the electrode at B covered a relatively wide area, and to the fact that the negative potential from the cut edges of the preparation was in part entering into the measurements. The central part of the strip was now replaced by a short water bridge (plate VII, fig. 9). A straight electrode tip with a small opening supported the water bridge at B. This arrangement insured a constant potential at B. Measurements were made as before. The growing

points A and C were both positive to B and in the A–C measurements, C was positive to A. The readings taken are given in table III. At no time was the difference between the expected difference of potential and the observed difference of potential greater than the limit of error. The results of this experiment, therefore, illustrate in a simple way the possible condition of summation of electromotive forces in cells of the leaf.

TABLE III

POTENTIAL DIFFERENCES IN MILLIVOLTS BETWEEN TWO GROWING POINTS ON OPPOSITE SIDES
OF THE LEAF, CONNECTED BY A WATER BRIDGE. (CONTACTS AS
SHOWN IN PLATE III, FIGURE 9)

A——B + −	B——C − +	EXPECTED PD A——C − +	OBSERVED PD A——C − +	DIFFERENCE IN MILLIVOLTS
+ 42.6	− 22.5	+ 20.1	+ 19.5	0.6
+ 43.6	− 21.5	+ 22.1	+ 22.2	0.1
+ 43.2	− 24.6	+ 18.6	+ 20.2	1.6
+ 47	− 24.5	+ 23.5	+ 22.6	0.9
+ 42.8	− 21.6	+ 21.2	+ 20.5	0.7
+ 50.2	− 29.5	+ 20.7	+ 21.8	1.1
+ 47.8	− 22	+ 25.8	+ 25.2	0.6

Discussion

The purpose of the foregoing experiments has been to present some of the general facts regarding the orientation, magnitude and variation of the inherent electric potentials in a leaf which possesses a system of growing points. The results show that the growing points on the leaf compose a dynamic system of individual electric polarities, whose variations in magnitude appear on the whole to be more or less independent of one another.

The observed electromotive force is without doubt an algebraic sum of a diversity of individual potentials of definite local origins. The potentials in the *Bryophyllum* leaf are fundamentally like those which occur in other polar growing structures, where the principle of summation of E. M. F. definitely applies, MARSH (12), LUND (10).

A study of the curves showing the simultaneous variations of potentials in different parts of the leaf and growing points forces the conclusion that in the leaf of *Bryophyllum* there exists a complex but characteristic system

of continuous electric currents of variable magnitude. The electrical energy of these currents is continuously dissipated and supplied from specific types of biochemical processes, probably cell oxidation (basal oxidation?) LUND (9).

If the leaf of *Bryophyllum* were immersed in an electrically conducting medium, then in a limited manner the approximate distribution of such a system of currents could be visualized. The simplest types of such a natural condition are found in hydroids and roots immersed in their natural medium In aerial structures the pathways of the "return circuits" are of course not indicated by such experiments as those in the present paper nor those in previous papers on the Douglas fir.

It has been suggested that the currents produced in the leaf may be photoelectric in origin. Many plants do produce photoelectric currents, in the production of which the activity of chloroplasts under the influence of light, plays a prominent part WALLER (15). These currents doubtless are related to plant metabolism in the process of carbohydrate manufacture by photosynthesis. The bioelectric currents in the experiments described in this paper are not photoelectric currents since they are produced in the dark as well as in light and since the points of highest potential are not the points of greatest photosynthetic activity in the leaf.

In a study of the structure of the growing points of a leaf of *Bryophyllum*, BEALS (1) found that the root and shoot arise from division of small phloem cells of the vein near the vegetative point in the leaf. This fact is particularly interesting since BOSE (2) has suggested that the phloem is the electromotively active tissue of a plant under stimulation. Perhaps the phloem is a specialized tissue which produces, in addition to the meristematic tissues the continuous bioelectric currents associated with growth processes.

Summary

1. The experiments which have been described show that a complex system of measurable, inherent, and continuously maintained electric potentials exist in the leaf of *Bryophyllum*.

2. In the experiments the distal part of the petiole was found to be positive to growing points and to selected points on the lamina.

3. Not all growing points are positive to any points on the lamina of the leaf. A point on the lamina close to a growing point may be positive to another growing point.

4. A growing point in the unstimulated condition was always positive to a point at the tip of the adjacent lobe.

5. The experiments indicate definitely that the total E. M. F. in a transverse strip isolated from the leaf and having a growing point at each end of

the strip is equal to the algebraic sum of a complex system of E. M. F.'s of individual cells of the strip. The results on the leaf of *Bryophyllum* extend and confirm the observations on roots, *Obelia*, and the Douglas fir.

LITERATURE CITED

1. BEALS, C. M.   An histological study of regenerative phenomena in plants. Ann. Missouri Bot. Gard. **10**: 369–384. 1923.

2. BOSE, J. C.   The nervous mechanism of plants. Longmans Green Co., London. 1926.

3. BUFF, H.   Ueber die Electricitätserregung durch lebende Pflanzen. Ann. Chem. und Pharm. **89**: 76–89. 1854.

4. BURDON-SANDERSON, J. S.   On the electromotive properties of the leaf of *Dionaea* in the excited and unexcited states. Phil. Trans. Roy. Soc. Lond. **173**: 1–56. 1883.

5. ELFVING, F.   Ueber eine Wirkung des galvanischen Stromes auf wachsende Wurzeln. Bot. Zeit. **40**: 256–263. 1882.

6. FRASER, M. T.   Parallel tests of seeds by germination and by electrical response. Ann. Bot. **30**: 181–189. 1916.

7. KUNKEL, A. J.   Electrische Untersuchungen an pflanzlichen und tierischen Gebilden. Pflüger's Arch. **25**: 342–379. 1881.

8. LUND, E. J.   The normal electric polarity of *Obelia*. A proof of its existence. Jour Exp. Zool. **36**: 477–494. 1922.

9. ——————.   Relation between continuous bioelectric currents and cell respiration. II. A theory of continuous bioelectric currents and electric polarity of cells. III. A theory of cell correlation. Jour. Exp. Zool. **51**: 265–290. 1928.

10. ——————.   Electric polarity in the Douglas fir. Pub. Puget Sound Biol. Sta. **7**: 1–28. 1929.

11. ——————, and KENYON, W. A.   Relation between continuous bioelectric currents and cell respiration. I. Electric correlation potentials in growing root tips. Jour. Exp. Zool. **48**: 333–357. 1927.

12. MARSH, G.   Relation between continuous bioelectric currents and cell respiration. IV. The origin of electric polarity in the onion root. Jour. Exp. Zool. **51**: 309–325. 1928.

13. MATHEWS, A. P.   Electrical polarity in the Hydroids. Amer. Jour. Physiol. **8**: 294–299. 1903.

14. MÜLLER-HETTLINGEN, J.   Ueber galvanische Erscheinungen an keimenden Samen. Pflüger's Arch. **31**: 193–214. 1883.

15. WALLER, J. C.   Photoelectric currents associated with activity of chlorophyll in plants. Ann. Bot. **39**: 515–538. 1925.

PLATE V

Plate V shows a horizontal section of a growing point from a leaf of *Bryophyllum* × 310. The growing point is located in the angle of a notch of the leaf and is embedded in leaf tissue. No branch of the vascular bundle reaches the growing point directly but branches are given off on each side of the notch. A few cells in the meristem tissue show mitosis. Many of the cells in the surrounding tissue show deposits of starch.
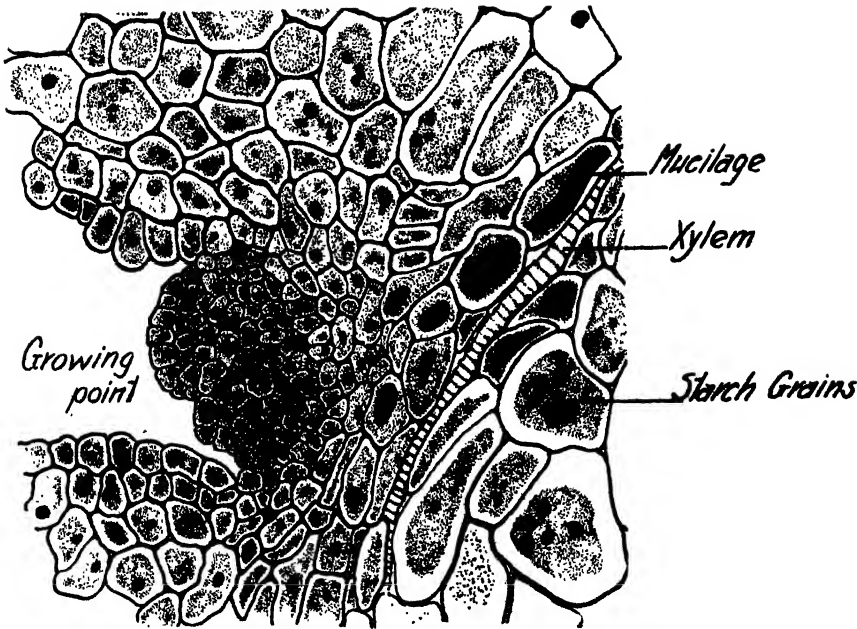
LUND AND BUSH—CORRELATION POTENTIALS
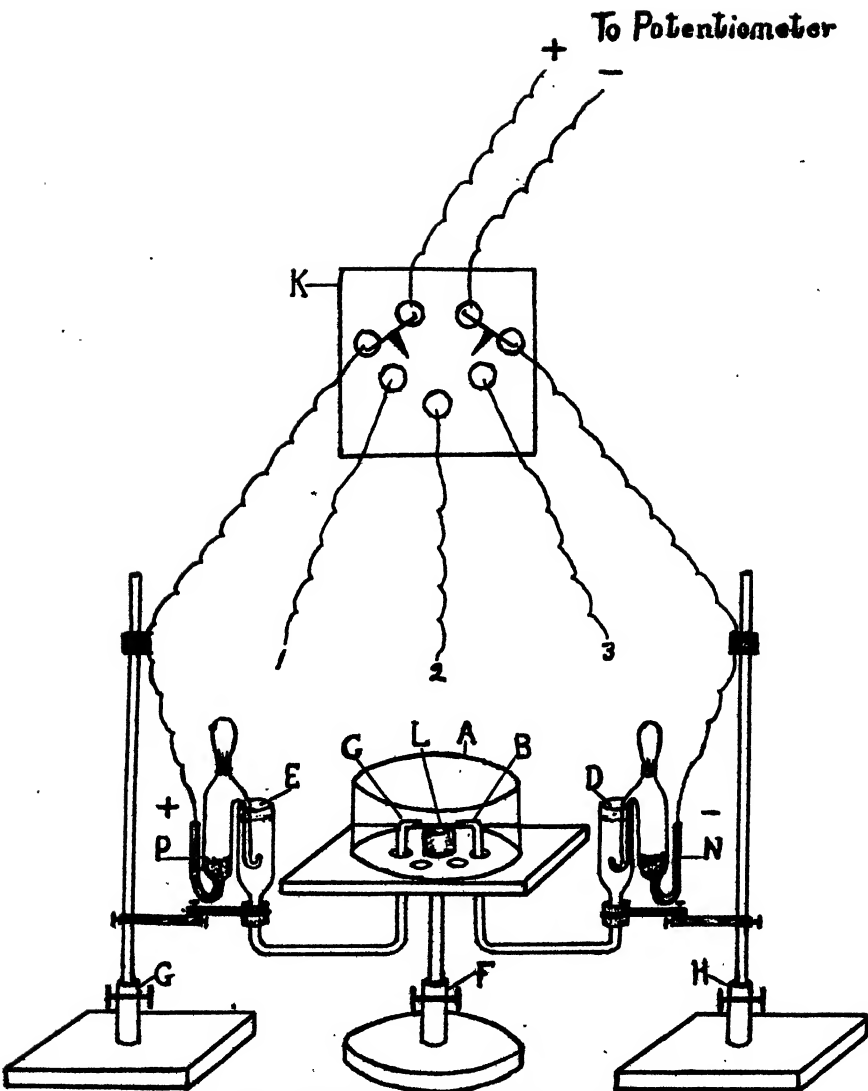
LUND AND BUSH—CORRELATION POTENTIALS

PLATE VI

Plate VI shows the arrangement of the apparatus with the wires leading to the key and to the potentiometer. Wires 1, 2, and 3 show possible electrode connections. These were used in the first five experiments when five electrodes were required to make the desired measurements.

PLATE VII

Plate VII shows the various leaf preparations and the position of the electrodes. In figs. 8 and 9 the directions of the currents are shown. The positive and negative signs refer to the outside circuit.
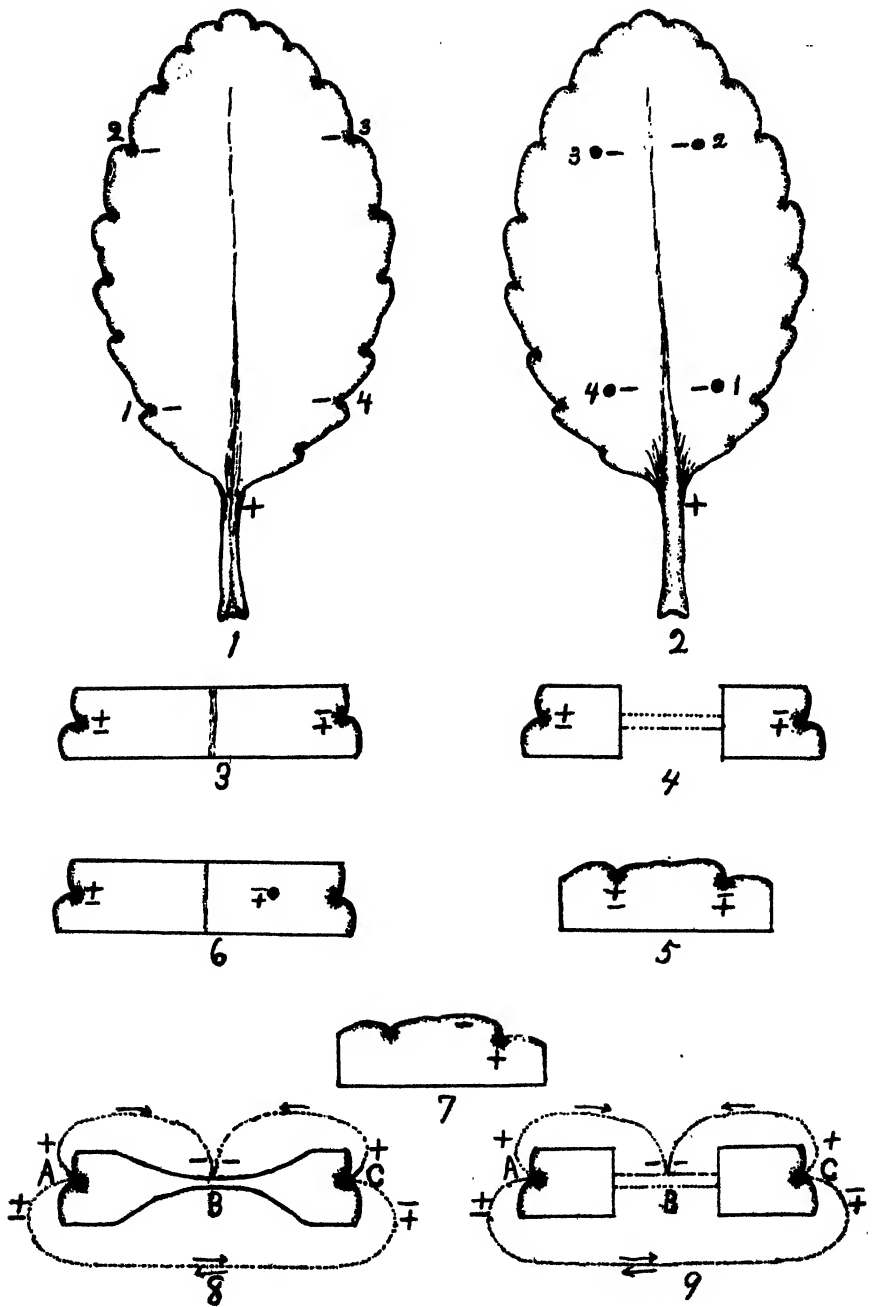
LUND AND BUSH—CORRELATION POTENTIALS

RESPIRATION OF APPLE TWIGS IN RELATION TO WINTER HARDINESS[1]

W. A. De Long,[2] J. H. Beaumont,[3] and J. J. Willaman[4]

(WITH SEVEN FIGURES)

The cold resistance of woody plants has hitherto been studied mainly from the static viewpoint. The problem has been attacked, in most cases, either by means of chemical analysis of whole ground tissues of hardy and tender plants or by microscopic analysis of the tissue structure. A minor amount of study has been directed towards microchemical determination of the distribution of food reserves within the tissues and the estimation of the seasonal changes in such food reserves. Some attention has also been devoted to measuring the amount of colloidal constituents and their degree of dispersion.

Life phenomena are, however, dynamic in character, and one of the most fundamental properties of living matter is its reaction to stimuli, or irritability. The fact of irritability may be demonstrated by the application of the most diverse agents, as anaesthetics, electrical shock, and changes in temperature. One of the most delicate means of showing that stimulation is so produced is by measurement of the changes which occur in respiration as evidenced by the rate of consumption of oxygen or of evolution of carbon dioxide. The present writers conceived the idea that respiration might throw some light on the phenomenon of winter hardiness in apple twigs. The present paper is a report of their findings.

It seems best to proceed directly with the description of the experiments, and to mention pertinent literature in connection with the discussion of our results.

Methods and apparatus

CO_2 PRODUCTION

The low temperatures desired were obtained by means of an ice-cream storage cabinet of the type described previously by us (3). Because of certain difficulties in temperature control encountered in the application of the air-bath method previously described, an oil bath was substituted in the second year of the work on the present problem. To this end the individual chambers of the cabinet were supplied with galvanized cans about one and

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one-half inches smaller in diameter than the chambers themselves and of such a height as to permit of the insertion into the top of the chamber of a cover carrying the necessary controls. These cans were filled with petroleum distillate. A brass pump of the type ordinarily used in water thermostats was placed in each chamber to maintain circulation. Heat was supplied as necessary by 110 volt, 125 watt electric heaters of the knife-blade type. The heaters were activated by means of the Harvey thermo-regulator relay system previously described (3, fig. 4). The temperature of the oil baths was thereby maintained constant within a range of about 0.1° C.

In all of the work presented here continuous aspiration was used, so as to avoid the effects of accumulated CO_2 previously emphasized (14). The carbon dioxide evolved was collected in absorption cells of the coil type already described (3, fig. 6), and absorbed in approximately 0.2 N standard NaOH. The semi-closed gas circuit system of circulation activated by an electric blower, previously described (3, p. 494) was used in the preliminary work of the season of 1926–7. Later, a one-way flow with compressed air was used.

In the case of the latter method the ingoing air was washed by passing through two consecutive absorption towers filled with 40 per cent. NaOH solution. These towers were fitted with absorption coils similar to those used in the measurement of the carbon dioxide evolved, with the exception that the central tube was lengthened at the lower end to the extent of about six inches in order that more pressure might be exerted upon the coil without forcing the air out at the bottom rather than up through the coil in the normal manner. This increase in length necessitated the use of larger containers for the coils, that is, about 1000 cc. The rate of flow was about 18 liters per hour through the washing tower, or 6 liters per hour for each of the three sets of absorption coils. The volume of the respiratory cells used being approximately 650 cc. when empty, or 450–550 cc. when filled with twigs, the rate of flow used assured a complete renewal of the gases in the system once every 10–15 minutes. For a carbon dioxide production of 100 mg. per kilogram-hour, which was about the maximum found in the present studies, the concentration of CO_2 in the atmosphere surrounding the twigs would not be in excess of 0.2 per cent.

After passing the washing towers, the air was led through a calcium chloride drying tower to prevent the freezing out of moisture at the low temperature points in the system. It was then passed over the samples of twigs and conducted thence to the absorption coils, two of which were placed in series in order to ensure complete removal of the evolved carbon dioxide from the outgoing gases. So far as possible all connections between the washing towers and the absorption cells were of copper tubing, the necessary rubber tubing joints being made as short as conveniently possible. The effl-

ciency of the washing towers, and the tightness of the joints were tested at frequent intervals during the course of the work. This was done by running the empty apparatus exactly as when in use for periods of 20 hours or more and determining the amount of carbon dioxide in the absorption cells at the end of that time. The average of all such blank runs was 0.04 mg. carbon dioxide per hour, which is well within the experimental error of the titration procedure for intervals of 10 hours or less.

The twigs were placed in copper respiratory cells similar to those of glass previously described by us (3, fig. 2) with the exception that the inlet tube was not coiled but simply passed down one side of the cell, across the bottom, and up the other to near the top, where it entered. The temperature within these cells was checked with a thermograph and found to be the same as that of the external bath, thus showing that the ingoing air was cooled to the desired extent during its passage through the straight inlet tube. As in the case of the glass containers, the outgoing gases were drawn off near the bottom of the cell. The top was closed with a heavy rubber stopper.

The samples usually consisted of 30 one-year twigs. These were placed in the respiratory cells as soon as possible after removal from the tree, the interval from tree to cell being about 30 minutes on the average and rarely more than 35 minutes. The cells were then immersed in the oil bath of the desired temperature, connected to the washing towers and absorption cells, and aspiration commenced immediately. Before titration, the carbonate in the absorption cells was precipitated with 10 per cent. $BaCl_2$ solution and the residual NaOH immediately titrated with approximately 0.1 N standard solution of HCl, using phenolphthalein. The results are uniformly expressed on the basis of the number of mg. of CO_2 produced per kilogram (fresh weight) of twigs per hour.

CHEMICAL ANALYSIS OF TWIGS

The twigs were ground in a motor-driven pencil sharpener immediately after removal from the designated storage chamber. The material, as soon as ground, was thoroughly mixed by shaking, and 25-gram samples withdrawn for analysis. These samples were weighed as quickly as possible on a balance accurate to 0.05 gram and immediately dropped into sufficient boiling 95 per cent. alcohol containing 0.2 gram calcium carbonate to make a final concentration of approximately 80 per cent. Heating was continued for one-half to three-quarters of an hour. The ground wood was subsequently extracted with alcohol in a Landsiedl extractor until a negative Molisch test was obtained. After making to volume the alcohol was removed from an aliquot by distillation under reduced pressure at a temperature of 40–50° C. After clearing with neutral lead acetate and removing the excess

lead with potassium oxalate, total and reducing sugars were determined on the aqueous filtrate. The QUISUMBING-THOMAS reduction method (11) was used and the reduced copper was estimated by permanganate titration after addition of saturated acid ferric sulphate solution according to the official methods of the Association of Official Agricultural Chemists. Acid-hydrolysable "starch" was determined on the alcohol-insoluble residue using the official method, except that reduction was carried out as indicated above.

RESPIRATION OF EXCISED TWIGS

In all cases in this paper the term "respiration" should be considered as synonymous with "CO$_2$ output," or "CO$_2$ evolution." On account of the very great experimental difficulties encountered by WILLAMAN and BEAUMONT (13) in their preliminary study of the respiration of apple twigs at low temperatures, in which measurement of the CO$_2$ production of twigs while still attached to the tree was attempted, it was decided to use only excised twigs in the present investigation. Such being the case it became of importance to determine to what extent the wounding of the twigs in their removal from the tree affected the subsequent CO$_2$ production. To this end uniform samples of twigs were collected at different times, divided into two portions, one of which was aspirated as collected while the twigs of the second portion were first cut in two, thus giving three cut surfaces per twig in place of one. Assuming that the effect of the second cut was practically identical with that of the first, one-half the difference between the amounts of CO$_2$ evolved by the first and second portions of the sample would be equal to the amount liberated from the cut surfaces and produced as the result of wound stimulation. This proved to be a relatively small and fairly constant fraction of the total, namely, 5–8 per cent. as shown by the data presented in table I.

EFFECT OF CHANGE OF TEMPERATURE ON THE RESPIRATION RATE.—When excised twigs are held at a constant temperature the rate of CO$_2$ evolution becomes fairly constant after 20 or 30 hours, but after 150 or 200 hours a steady decline is noticed. In this paper, however, we will deal with time periods of less than 30 hours, and in this case we do not have an equilibrium rate.

Of the first two samples of twigs investigated, the one (A) was held at $+6°$ C. and the other (B) at $-2°$ C., and it was found that while the respiratory level of (B) became relatively constant after the first few hours of aspiration, that of (A) continued to show a very appreciable falling off in rate even after 200 hours. It was then decided to reverse the temperatures of the two samples in order to determine the effect of small temperature changes upon the intensity of respiration at this low level. The result

TABLE I

EFFECT ON CARBON DIOXIDE OUTPUT OF CUTTING TWIGS

VARIETY	No. OF CUTS	DATE	TOTAL CO_2	CO_2 PER KG.	DIFFER- ENCE	TOTAL CO_2, DUE TO ONE CUT
			mg.	mg.	per cent.	per cent.
Duchess	One	Feb. 29	194	916		
	Two		197	1019	+ 11.2	5.6
Florence	One	March 4	195	1269		
	Two		250	1446	+ 14.0	7.0
Hibernal	One	March 14	134	789		
	Two		152	919	+ 16.5	8.25
Hibernal	One	March 24	409	2601		
	Two		449	2948	+ 13.4	6.7
Hibernal*	One	March 31	58	378		
	Two		66	416	+ 10.2	5.1

Mean difference (per cent.)............ 6.53 ± 0.26

* Aspiration at − 10° C. All the preceding determinations were made at + 6° C.
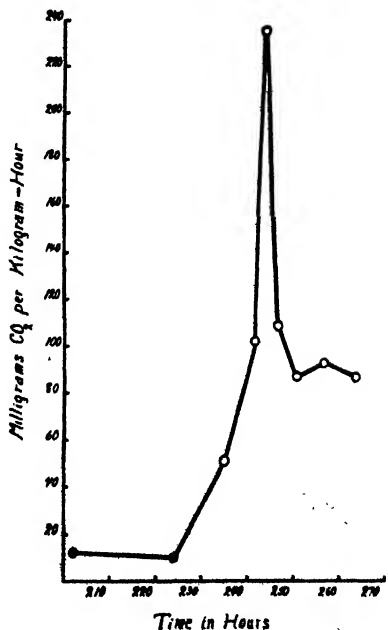
FIG. 1. Output of CO_2 by apple twigs at 6° C. after storage at − 2° C. Solid dots represent measurements at − 2° C., and circles those at 6° C.

of changing sample (B) from $-2°$ C. to $+6°$ C. is represented graphically
in fig. 1, a conclusive demonstration that small upward variations in tem-
perature within this range were capable of greatly accelerating this respira-
tory rate. On the other hand, changing the temperature of sample (A)
from $+6°$ C. to $-2°$ C. merely caused the rate of respiration to fall to a
level practically identical with that previously exhibited by sample (B) at
that temperature.

These results were repeatedly confirmed. Fig. 2 contains representative
curves of respiration over periods of 90 hours, when the temperature of
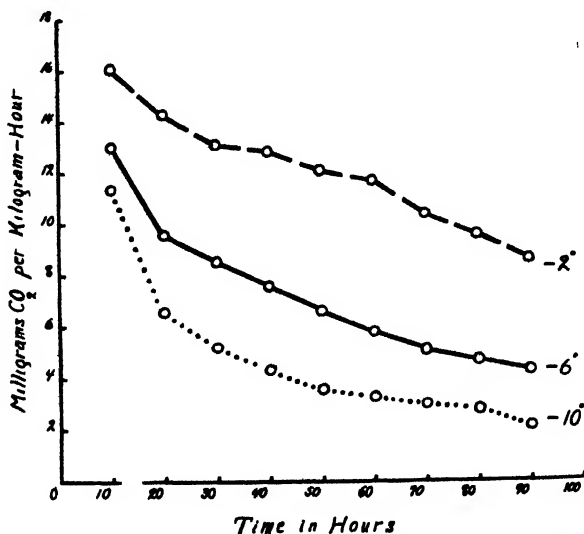
FIG. 2. Output of CO_2 by apple twigs at three lower temperatures after storage
above $0°$ C.

the respiration period is *lower* than that of the previous period. It will be
seen that there is an immediate falling off in CO_2 output, with a tendency
to assume a level after some time. On the contrary, when the temperature
of the experimental period is *higher* than that of the previous period, the
CO_2 production always rises to a peak within a few hours, and then declines
to a more or less constant value. Furthermore, the lower the previous tem-
perature, the greater is the peak production. This is illustrated in fig. 3.

If this phenomenon obtains generally, it is obvious that, in attempting
to determine the respiratory rate of plant material, cognizance must be
taken of its previous temperature history. We thought that one way of
determining whether this is the case would be to take samples at intervals
during the winter, noting the temperatures during the previous 24 hours,
and measuring the rate of CO_2 production at $6°$ C., which temperature
would be higher than that of the orchard for all samples.
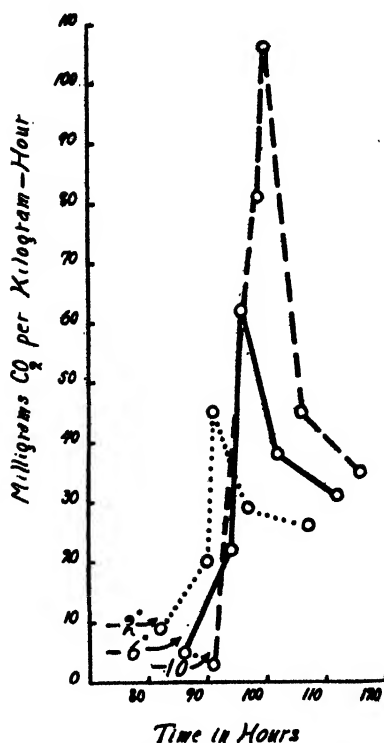
Fig. 3.   Output of CO_2 by apple twigs at 6° C. after storage at −10° C., −6° C., and −2° C., respectively, for about 90 hours.

A series of 64 samples was taken from Dec. 21, 1927, to April 15, 1928. After placing in the respiration chamber the CO_2 was swept out continuously and measured every one or two hours until the peak was passed. The data will be presented in two groups.   The first involves 8 samples taken during a period of unusual change in temperature.   They are presented in fig. 4.   The maximum rate of CO_2 production is plotted for each sample.   These curves are clearly the mirror images of each other.   This means, of course, that the lower the temperature previous to the period at 6° C., the greater is the CO_2 production at that temperature.   The second group includes all the samples secured during the whole winter period. The results were analyzed statistically.

The biometrical constants determined are summarized in table II.   In this table the letters A, B, C, D, E, F, G, and R have the following significance:

A = the temperature in the orchard at the time of collection.
B = the maximum temperature attained on the day of collection.

C = the minimum temperature attained on the day of collection.

D = the rate of carbon dioxide production (in milligrams per kilogram-hour) during the first hour of aspiration.

E = the maximum rate of carbon dioxide production attained (milligrams per kilogram-hour).

F = the total amount of carbon dioxide produced in the first twenty-four hours of aspiration (milligrams per kilogram).

G = the rate in milligrams per kilogram-hour for the second and third hours of aspiration.

R = the total amount (F) of carbon dioxide produced in twenty-four hours minus the amount produced in the first hour (D) in milligrams per kilogram.
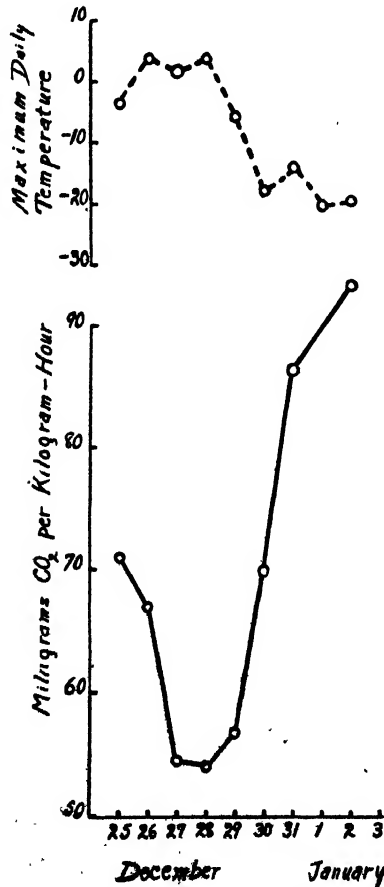
FIG. 4. Effect of orchard temperatures on rate of CO_2 output by apple twigs.

It should be noted that in the case of the temperature values a uniform increment of thirty degrees was added. By this means all temperature readings were raised to positive values.

In the calculation of the biometrical constants reported the following formulae were used:

Mean = $S(X)/N$.

Standard deviation = $\sqrt{S(X)^2/N - \overline{X}^2}$.

Coefficient of correlation = $\dfrac{S(XY)/N - \overline{XY}}{s_X s_Y}$.

Coefficient of correlation of a part to the remainder of the whole,* or

$$r_{XY} = \dfrac{S(XY) - S(X)^2/N - \overline{XY}}{s_X s_Y}.$$

(Note: This was used only in the case of the correlation coefficient for DR.)

Probable error or mean = $0.6745 \cdot s_X \sqrt{N}$.

Probable error of correlation coefficient = $0.6745 \cdot (1 - r^2)/\sqrt{N}$.

In these formulae the letters have the significance: S = summation, X = the values of one variable, Y = the values of the other variable, N = the number of observation, r = the coefficient, s = the standard deviation, $\overline{X}$ and $\overline{Y}$ the means of the respective variables.

TABLE II

RELATION BETWEEN CO_2 OUTPUT AND PREVIOUS ENVIRONMENTAL CONDITIONS.
FOR EXPLANATION OF SYMBOLS SEE TEXT

MEAN	STANDARD DEVIATION	CORRELATION COEFFICIENT
A 23.127	7.144	AD .... -0.049 ± 0.084 AE .... -0.458 ± 0.067 AF .... -0.424 ± 0.069
B 27.195	7.342	BD .... -0.078 ± 0.084 BE .... -0.432 ± 0.069 BF .... -0.399 ± 0.071
C 19.469	7.458	CD .... -0.121 ± 0.083 CE .... -0.525 ± 0.061 CF .... -0.490 ± 0.064
D .... 36.420 ± 1.252	14.764	DE .... $+0.751 \pm 0.037$
E .... 53.456 ± 1.140	13.528	GE .... $+0.857 \pm 0.022$
F ....1175.453 ± 24.351	288.809	DR .... $+0.745 \pm 0.038$

* The writers are indebted for permission to make use of this formula to the origi-nator, Dr. J. A. HARRIS, late Professor of Botany, University of Minnesota.

The results obtained support the conclusion that the rate of respiration is proportional to the increase in temperature to which the twigs are subjected when they are brought from the orchard to the respiration chamber. In addition, another very interesting and important relation is revealed. Thus it is found that a very good correlation exists between the rate of carbon dioxide production in the first few hours of aspiration and the maximum carbon dioxide production attained. The existence of this correlation indicates that in the comparison of the respiration of hardy and tender varieties of apple it is necessary to make determinations during only the first four or five hours.

Of the three temperature values, the minimum gives a better correlation with both the initial and maximum rates, and also with the total production of carbon dioxide, than either the temperature at the time of sampling or the maximum temperature of the day. This was to be expected from the observations above on the influence of extent of elevation of temperature upon carbon dioxide evolution. This is further supported by the fact that the sampling temperature, which was lower than the daily maximum (since the collections were made in the morning), shows a better correlation with both the maximum rate and the total for 24 hours than does the maximum. It is noteworthy also that the correlation of all temperatures with the *maximum rate* of carbon dioxide production is higher than with the initial rate or the total in twenty-four hours. In other words, so far as the effect of temperature is concerned the maximum rate is the most significant measurement. However, as shown by r_{DE} there is a significant relation of the initial to the maximum rate, a relation which it is not unlikely a refinement of technique would show to be more pronounced than is here indicated. Thus, under the experimental conditions adopted, the measurement of the initial rate has been the least accurate of any of the observations made, by reason of the facts that the initial hour was, as a rule, the shortest time interval over which measurements were made, that the amount of carbon dioxide evolved during the first hour was usually less than that given off at any subsequent hour under observation, and that an hour or two was required to bring the twigs to 6° C. In addition, the maximum rate measured is at best only an approximation, especially when the rise and fall in carbon dioxide production is rapid. Early experience showed that a maximum rate could be expected to occur in the vicinity of the 8th to 10th hours. Accordingly, readings were taken more frequently up to this point than later; nevertheless, no claim can be made that the true peak of the curve was more than approximately located.

It is our opinion, therefore, that the actual correlation between the initial rate and the maximum attained is considerably higher than that

actually found, and that a refined technique might make possible the use of a shorter observational period, possibly as low as four hours.

Before offering an explanation of the above results, it might be desirable to summarize them in concise form: (1) When twigs are brought from a lower to a higher temperature for the measurement of CO_2 production, the latter rises to a peak and then declines to a more or less constant value. (2) The lower the previous temperature, the greater is the peak value. (3) The latter principle holds not only for excised twigs in storage, but for twigs on the trees.

Two possible explanations of the above phenomena occur to us. They may be stated as follows: (1) When plant tissues are subjected to the action of low temperatures very considerable changes occur in the tissue-water equilibrium. It is a generally accepted fact that at temperatures below $0°$ C. ice crystals are formed, not within the individual cells, but in the adjoining intercellular spaces. This withdrawal of water from living tissues must occasion far-reaching disturbances of cellular chemical equilibria and of physical organization. It is accordingly suggested that the apparent increase in carbon dioxide production observed to follow the elevation in temperature of apple twigs from levels below to levels above $0°$ C. is primarily the result of a temporary stimulation of carbon dioxide production resulting from that derangement of the tissue-water equilibrium. Examples of such low temperature effects upon chemical equilibria within living tissues are the well-known accumulation of sugar in potato tubers, and the increased production of lactic acid in the living muscle of the frog at $-2°$ to $-3°$ C. The postulated stimulation of carbon dioxide production in apple twigs may then be explained as the result of the increased effective concentration of respiratory substrate within the cells following withdrawal of water to the intercellular spaces. Another contributing factor may be increased hydrogen-ion concentration following accumulation of carbon dioxide.

The observed differences between twigs from hardy and from tender varieties of the apple may, on the basis of the above hypothesis, be related directly to the possession by the former of hydrophilic colloids in greater amount, or of more intense imbibitional capacity. On this assumption the water loss from the cells of hardy twigs would be less, and, consequently, the disturbance of normal cellular equilibria correspondingly less, than in the cells of the tender twigs. That is to say, the hardy twigs are more stable or less irritable than twigs of more tender varieties. Further, the return of water to the cells and the resumption of more or less normal metabolism would be more rapid in the case of the former than in that of the latter, a factor conceivably of importance in determining the survival of tissues under rigorous climatic conditions.

(2) Another possibility is that we are dealing here simply with the solution of CO_2 in the twig sap. This gas is increasingly soluble in aqueous media at decreasing temperatures. Therefore, the peak of CO_2 evolution represents the greater solubility of the gas at the previous lower temperature than at the temperature of measurement. Conversely, when measurement is made at a lower temperature than that of the storage, the resultant time curve represents the establishment of an equilibrium between the solubility of CO_2 in the tissue and its diffusion into the outer atmosphere. Evidence favoring this conception is offered in the article following this (15). It consists in measuring the dissolved CO_2 of twigs by submerging the latter in boiling alcohol in a closed system and distilling out the CO_2 under vacuum. The CO_2 is completely removed in an hour or so, and it is demonstrable that the amount of the dissolved gas varies inversely with the temperature, and that this is roughly of the same magnitude as that contained in the peak of the curves in this paper. This method had not been devised at the time the measurements in this paper were made; hence, it is utilized here merely as a possible explanation of the observed facts.

RELATION OF CO_2 PRODUCTION TO WINTER HARDINESS

A number of varieties of apple known to be of varying degrees of hardiness have been compared in respect to the evolution of CO_2 at 6° C. Only samples collected on the same day and so far as possible subjected to identical conditions subsequent to removal from the tree have been compared directly. Twigs obtained from several different sources and possessing correspondingly variant histories as noted below were utilized.

On January 19, 1928, samples of one-year twigs of Hibernal, Duchess, Charlamoff, McIntosh, Haralson and Jonathan were collected at the University Fruit Breeding Farm. These twigs were taken between the hours of 11 A. M. and 2 P. M., tied in bundles, and kept at a temperature as near as possible to that obtaining in the orchard on that date until received at University Farm, when they were placed in a cold storage room in which the temperature was maintained at 0° C. ± 2°. Samples were taken for measurement at intervals during the period January 21–31. That the respiratory power of the material did not vary greatly during this period is indicated by the results obtained with Hibernal (table III). The results obtained in the comparison of these different varieties are presented in fig. 5, the curves in which are based either upon single determinations or the average of two or more determinations, as follows:

BEAUMONT and HILDRETH have devised a system of evaluating the amount of winter injury in apple twigs, by the degree of browning in certain specific regions of the twigs. By means of this they have been able to

TABLE III

CONSTANCY OF CARBON DIOXIDE EVOLUTION BY HIBERNAL TWIGS COLLECTED ON JANUARY 19TH, 1928

DATE ANALYZED	NUMBER OF TWIGS	WEIGHT OF TWIGS	CARBON DIOXIDE PER KILOGRAM-HOUR AT 6° C.						
			AT 30 MIN.	AT 3 HRS.	AT 7 HRS.	AT 10 HRS.	AT 15 HRS.	AT 20 HRS.	AT 30 HRS.
			mg.	mg.	mg.	mg.	mg.	mg.	mg.
Jan. 24.........	30	137 g.	22	32	39	41	40	39.5	38.0
Jan. 26.....	40	154 g.	22.5	31	39	41.5	42.5	42.7	....
Jan. 31.........	30	135 g.	24	36	38.5	39	39.2	38.5	36.5
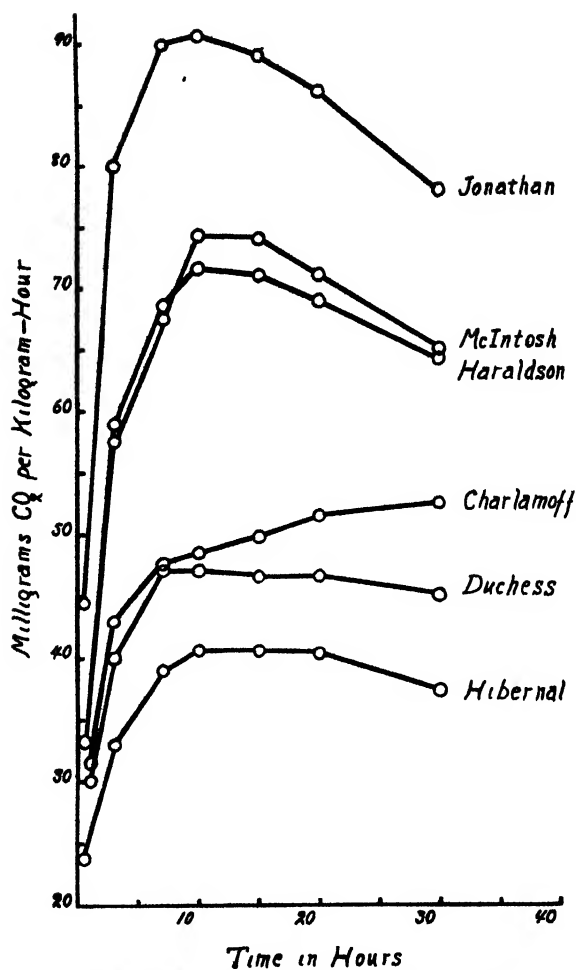
FIG. 5.   CO₂ output of 6 varieties of apple twigs of varying degrees of winter hardiness.

TABLE IV
DETERMINATIONS USED IN FIG. 5

VARIETY	NO. SAMPLES	DATE DETERMINED	AVERAGE WEIGHT OF SAMPLES
Haralson	3	Jan. 24, 26, and 31	129
Duchess	1	Jan. 21	126
Charlamoff	2	Jan. 21 and 24	131
McIntosh	2	Jan. 27 and 29	114
Haralson	3	Jan. 26, 27 and 29	91
Jonathan	1	Jan. 31	136

assign definite numerical hardiness values by which the varieties may be compared. Table V contains their values.* The lowest values indicate least browning and hence greatest hardiness.

It will be seen that the order of magnitude of CO_2 production in fig. 5 is the same as the order of hardiness for these 6 varieties. Thus the hardiest varieties evolve the least CO_2 under the conditions of measurement, and the tenderest evolve the most. Not only is the serial order the same, but the quantitative relations are rather regular; thus the hardiness values of the 6 varieties in fig. 5 from bottom to top are 6, 8, 10, 12, 16, and 24.

TABLE V

BEAUMONT AND HILDRETH'S HARDINESS VALUES FOR APPLE VARIETIES

VARIETY	AVERAGE BROWNING FOR 4 YEARS— 1924–1928	AVERAGE BROWNING FOR 3 YEARS— 1924–1927
Dolgo	2.0	2.3
Hibernal	7.0	6.0
Oldenburg (Duchess)	7.0	7.7
Charlamoff	....	10.0
Patten	11.8	11.0
Haralson	14.5	12.0
Wealthy	15.5	12.7
Anisim	....	14.0
McIntosh	21.2	15.7
Minnehaha	20.5	17.0
Fameuse	22.2	16.3
Cortland	....	18.3
Salome	....	19.3
Delicious	25.0	20.0
Wolf River	26.8	21.0
Winesap	29.0	22.0
Jonathan	28.5	23.7
Ben Davis	28.5	24.0
Sugar Loaf	....	24.7
King David	....	25.7
Lansingburg	....	25.7
Yellow Belleflower	....	30.3
Tompkins King	....	36.3

These results are contrary to the preliminary measurements of WILLAMAN and BEAUMONT on Charlamoff and Delicious (13), in which the hardier seemed to emit more CO_2 than the tender. The explanation is that at the time those measurements were made we did not realize the very great effect

* Although these values have not been hitherto published, a description of the method of arriving at the values is given by B. H. WILSON in Sci. Agr. 10: 598–606. 1930.

of the previous temperature. The two varieties were measured alternately and no attention paid to the temperature previous to each measurement. We can see now that this fact could completely jeopardize any varietal differences.

Since examination showed that considerable winter injury had already occurred in certain of the samples reported on above, as indicated by the "browning test," particularly in McIntosh, Haralson, and Jonathan, it appeared possible that the increased CO_2 production shown by these varieties over that of the more hardy sorts might be the result of this winter injury rather than due to any inherent difference in respiratory power. Accordingly, samples of Red Duchess and Delicious, in which practically no frost injury could be detected, were obtained from the tubbed breeding stock in the cold storage cellar at the Fruit-breeding Farm. These samples were taken on February 10th, held in the cold cellar of the Horticultural Build-
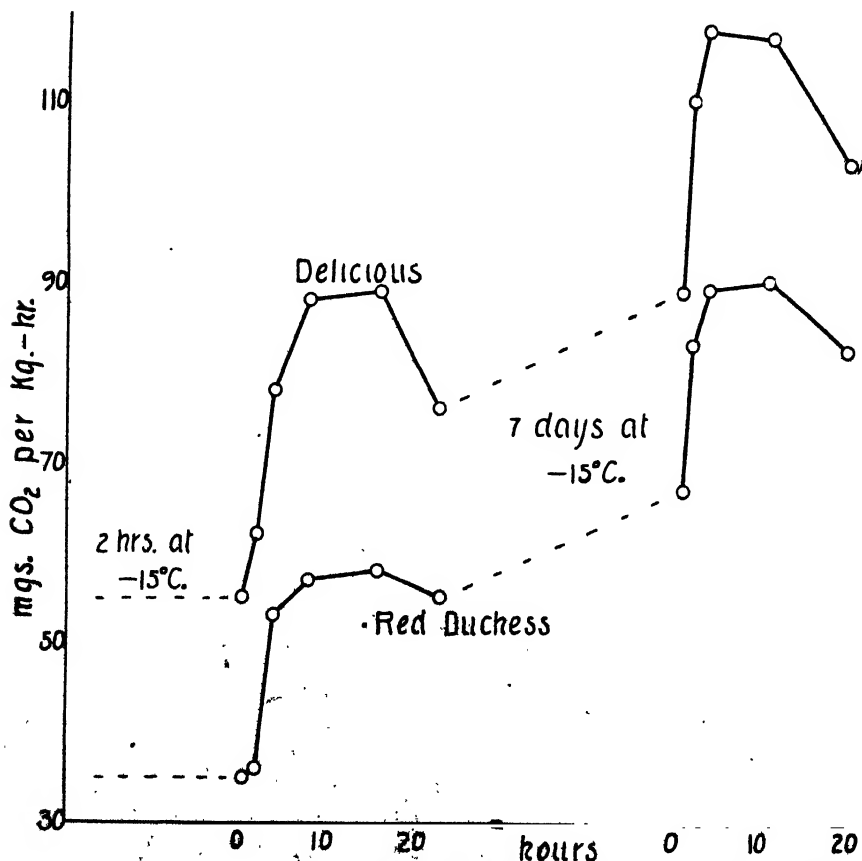
Fig. 6. Accumulation of CO_2 in apple twigs stored at $-15°$ C. These curves also illustrate a varietal difference due to hardiness, Duchess being hardier.

ing at University Farm over-night at a temperature of about 5° C., and then, on the morning of February 11th, subjected to a temperature of – 15° C. for two hours before aspiration was commenced. The temperature changes occurring subsequent to removal of these twigs from the long-continued even temperature of the storage cellar at the Fruit Farm probably account for the relative high rate of carbon dioxide production found in both these samples. No more of this material was available. Consequently, after twenty-four hours' aspiration the twigs were replaced in the storage room at – 15° C., where they were held for seven days. At the end of this time they were again aspirated at 6° C. for a second twenty-four hour period. The results obtained are shown in fig. 6. It will be noted that the respiratory level has been raised by holding at – 15° C. in the case of both varieties, but that the *relative* difference between the hardy Duchess and the tender Delicious has not been materially altered. It is therefore considered that the differences shown in fig. 6, as well as in fig. 7, are due to inherent varietal qualities rather than to low temperature injury.

Further data respecting the relative rates of carbon dioxide production in hardy and tender varieties were obtained on materials of a more restricted range in hardiness, none of which showed appreciable injury. Such was the case for the comparison of Patten with Duchess of Oldenberg, and Florence with Hibernal, the former variety in each instance being the less hardy. Samples were taken on 4 different dates for the first pair and on 5 for the second. The data for the various samples were averaged, and
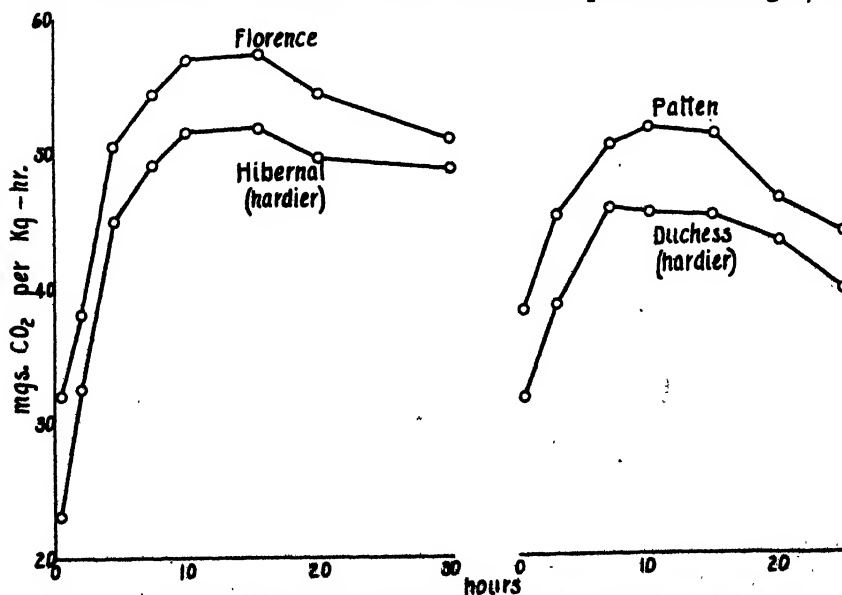
Fig. 7. Comparison of CO_2 output of varieties differing in hardiness.

are presented in fig. 7. The mean difference with its probable error, the standard deviation, and the deviation from a random distribution were calculated for these data, and found to be as follows:

TABLE VI

STATISTICAL STUDY OF DIFFERENCES BETWEEN NON-HARDY AND HARDY APPLES

VARIETIES	MEAN DIFFERENCE	STANDARD DEVIA-TION OF DIFFERENCE	DEVIATION FROM RANDOM DISTRI-BUTION
Florence–Hibernal	± 5.4903 ± 0.6494	5.8605	+ 9.5 ± 1.8777
Patten–Oldenburg	± 5.9425 ± 0.4088	4.0408	+ 18.0 ± 2.2483

For the first two varieties the mean difference is thus seen to be more than eight times its probable error, while the deviation from a random distribution is more than five times as great as the probable error. In the case of Patten and Duchess the differences are even more significant, the corresponding values being nearly fifteen and more than eight times their probable errors, respectively.

We were able to make still another comparison of varieties during the winter of 1928–9. Eleven varieties were secured at the University of Minnesota Fruit Breeding Farm on Dec. 28, stored at $-15°$ C., and analyzed during the next few days. The data secured are given in table VII. Before placing in the respiration chamber the twigs in series A were kept at $20–23°$ C. Those in series B were placed in the chamber directly from the storage chamber. Because of the small number of samples it was not considered feasible to calculate the ordinary coefficient of correlation, hence that by rank was used, according to the formula $r = 1 - 6 \, (SD^2_k)/n^3 - n$.

Although in series A the agreement between hardiness and CO_2 production is only fair, that in series B is very good, and they both indicate the same relation in the samples of the previous winter. We therefore feel justified in concluding in general that the hardier an apple variety, the lower its intensity of CO_2 production under the experimental conditions described.

The question immediately arises as to whether the solubility of CO_2 in the twig sap can be invoked here also in explanation of these facts. Evidence is presented in the following paper by WILLAMAN and BROWN (15) that such may be the case: these varieties differ in their content of dissolved CO_2 in the same order as their hardiness, and in quantities comparable to the peaks in CO_2 curves shown above.

TABLE VII

CO₂ OUTPUT OF APPLE TWIGS AT 6° C. DURING FIRST SIX HOURS

VARIETY	HARDINESS VALUE*	SERIAL ORDER OF HARDINESS	A THAWED BEFORE PLACING IN THERMOSTAT**		B NOT THAWED	
			CO₂ PER KG.	SERIAL ORDER	CO₂ PER KG.	SERIAL ORDER
			mg.		mg.	
Hibernal	6	1	88	3	106	2
Duchess	8	2	80	1	98	1
Patten	11	3	105	8	133	4
Haralson	12	4	97	7	146	7
Wealthy	13	5	90	5	138	6
McIntosh	16	6	91	6	137	5
Fameuse	16	7	88	4	126	3
Wolf River	21	8	81	2	154	8
Jonathan	24	9	128	10	175	9
Sugar Loaf	25	10	119	9	212	10
Lyman Perfect***			93		....	

* BEAUMONT and HILDRETH.

** Kept in laboratory for 30 minutes.

*** Not listed by BEAUMONT and HILDRETH.

Coefficient of correlation (by rank) between tenderness and CO₂ output:—

$$r_A = + 0.478 \pm 0.172$$
$$r_B = + 0.818 \pm 0.074$$

CHANGES IN COMPOSITION OF EXCISED TWIGS

It seemed desirable to determine to what extent chemical changes were occurring in the twigs under the experimental conditions used. Analytical results on a number of samples under various storage conditions are given in table VIII. The averages of the changes in composition at each temperature were calculated to a basis of 10 day periods, and are given in table IX.

No striking changes in composition occurred. Nevertheless, certain tendencies are apparent, which may be summarized as follows: (1) The moisture losses are not great. (2) With aspiration, there is a slight gain in starch and a loss in sugars. (3) Without aspiration there is not much change in starch, and a gain in sugars. (4) In other words, the accumulation of CO_2 in the air around the twigs, and hence its presumed accumulation in the tissues, causes an accumulation of sugar in the tissue, either because of reduced respiration, or because of a changed equilibrium between starch and sugar.

TABLE VIII

CHANGES IN COMPOSITION OF EXCISED TWIGS DURING STORAGE AT LOW TEMPERATURES

DATE	MOISTURE	REDUCING SUGARS		TOTAL SUGARS		ACID-HYDROLYZABLE "STARCH"		REMARKS
		FRESH	DRY	FRESH	DRY	FRESH	DRY	
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	
Feb. 3	46.0	1.52	2.82	5.47	10.93	10.8	20.0	At time of collection
	40.7	2.12	3.58	6.67	11.26	12.0	20.2	After 18 days without aspiration at −10°
	46.4	1.28	2.39	4.40	8.22	11.5	21.5	After 21 days aspiration at −2°
	41.2	1.32	2.25	4.51	7.67	12.6	21.4	After 21 days aspiration at −6°
	45.4	1.31	2.40	4.27	7.83	12.0	21.5	After 21 days aspiration at −10°
Feb. 26	43.9	1.26	2.25	4.19	7.47	12.3	21.9	At time of collection
	42.3	1.43	2.48	4.80	8.33	12.7	22.0	After 6 days without aspiration at −6°
	42.7	1.40	2.45	4.88	8.53	12.6	22.1	After 6 days without aspiration at −10°
	43.6	0.99	1.76	3.55	6.30	12.5	22.2	After 30 days aspiration at −2°
	41.9	1.28	2.20	4.58	7.88	13.0	22.4	After 30 days aspiration at −6°
	44.5	1.14	2.06	3.97	7.16	12.0	21.7	After 30 days aspiration at −10°

TABLE VIII—(*Continued*)

Date	Moisture	Reducing sugars		Total sugars		Acid-hydrolyzable "starch"		Remarks
		Fresh	Dry	Fresh	Dry	Fresh	Dry	
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	
March 29	47.9	1.28	2.46	4.43	8.51	11.1	21.4	At time of collection
	45.2	1.46	2.66	5.30	9.90	11.5	21.1	After 4 days without aspiration at −2°
	46.8	1.43	2.69	4.93	9.28	11.5	21.6	After 4 days without aspiration at −10°
	45.2	1.15	2.10	4.68	8.55	12.0	22.0	After 8 days aspiration at −2°
	47.0	1.17	2.21	4.27	8.06	11.6	22.0	After 8 days aspiration at −10°
April 6	48.9	1.12	2.19	4.05	7.93	11.5	22.5	At time of collection
	44.6	1.58	2.85	5.97	10.78	11.1	20.1	After 8 days without aspiration at −2°
	47.2	1.08	2.05	4.68	8.86	10.8	20.5	After 8 days without aspiration at −10°
April 16	45.4	1.20	2.20	3.58	6.57	10.1	18.5	At time of collection
	50.0	1.89	3.79	7.17	14.36	11.2	22.4	After 13 days without aspiration at −2°
	49.5	1.22	2.42	4.64	9.20	10.8	21.5	After 13 days without aspiration at −10°

TABLE IX

CHANGE IN COMPOSITION OF APPLE TWIGS PER 10 DAY PERIODS UNDER VARIOUS STORAGE CONDITIONS

CONSTITUENTS	DRY MATTER BASIS					
	WITH ASPIRATION			WITHOUT ASPIRATION		
	$-2°$ C.	$-6°$ C.	$-10°$ C.	$-2°$ C.	$-6°$ C.	$-10°$ C.
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Starch	+ 0.54	+ 0.44	+ 0.47	− 0.30	+ 0.11	+ 0.16
Reducing sugars	− 0.27	− 0.15	− 0.19	+ 0.85	+ 0.38	+ 0.26
Total sugars	− 0.43	− 0.53	− 0.69	+ 3.01	+ 0.86	+ 1.46
Moisture	− 1.50	− 1.50	− 0.43	− 2.70	− 2.50	− 1.20

These results cannot be emphasized strongly, because of the limited number of samples and the rather small differences in composition. Nevertheless, they are suggestive and are included here partly to augment the very sparse published analyses of apple twigs.

Discussion

It has been shown that storage of apple twigs at lower temperatures, followed by their elevation to a higher temperature, gives rise temporarily to a considerable increase in rate of CO_2 output. Furthermore, the rate at the higher temperature is greater, the lower the previous temperature of storage. The same facts obtain when twigs are not taken from storage, but measured immediately after taking from the tree. It has also been shown that the hardier varieties respond to such variations in temperature to a less degree than the tenderer. We have shown in another paper that the CO_2 dissolved in the twig tissue can probably account for most of this temporary flush, and that the quantity of this dissolved CO_2 is proportional to the tenderness of the variety.

These findings agree with many of the observations found in the literature of plant respiration. In 1885 DEHÉRAIN and MAQUENNE (2) first called attention to the CO_2 dissolved in plant sap and to its possible effect on respiration measurements. Later MAQUENNE (6) attempted to remove this CO_2 by evacuation, but was only partly successful. SIMON (12) has measured the CO_2 production of excised twigs of beech, red oak, basswood, and horse chestnut. The twigs were brought from various outdoor temperatures to 22.5° C. for measurement, and SIMON noted that the lower the previous temperature, the greater was the production of CO_2 during

the early part of the measurement period.   This effect was so pronounced that he extended his measurements over a period of seven days in order to avoid that temporary flush.

Increasing the temperature within the range of normal growth has frequently been observed to produce a temporary increase in CO_2 evolution. A very definite effect of this nature was early noted by MÜLLER-THURGAU (8) in potato tubers, and has since been reported for many other types of tissue, as, for example, etiolated shoots of *Vicia faba* (PALLADIN, 10), gladiolus bulb (ZALESKI, 16), and possibly sweet potato tubers (JOHNSTONE, 5) and bananas (OLNEY, 9).

In the case of the sweet potatoes the tubers were stored at 15–20° C. for some time and then brought to 25° C. for CO_2 measurement.   The curves show a peak at about the tenth day, followed by a decline to a constant level for another 20 or 30 days.   The author says, "The slight increase in respiration shown by the curves on the fourth to seventh days is undoubtedly due to transferring the sweet potatoes to the higher temperature for respiration determination, but cured sweet potatoes do not respond so readily to such a change as uncured."   This statement does not, of course, explain the temporary flush of CO_2.   We cannot readily see how this peak could be due to dissolved CO_2 accumulated at the lower temperature, because it requires seven to ten days for dissipation.   In the case of the bananas the respiration curves have a shape very similar to those of apple twigs; but again the peak comes during the second to fourth days. It is possible that in each case we are dealing with the slow evolution of dissolved CO_2, and that the thickness of the tissue, its wateriness and its solvent power for CO_2 determine the time required for an equilibrium to be established at a higher temperature.   It might be mentioned here that, when twigs are submerged in boiling alcohol and evacuated, an hour and a half is required for complete removal of the dissolved CO_2; hence 5 to 10 days might be a plausible period for this purpose in a tissue like sweet-potato tubers, with only the normal circumstances for diffusion of the gas.

Respiratory activity in winter cereals has been more recently studied by GOVOROV (4) and by MARTIN (7).   The former found that in winter wheat and rye the amount of CO_2 liberated was 300 and 800 mg. per hour per kilo of dry weight at 3° C.   He also found that spring wheat and spring rye, under the same conditions, gave off considerably larger quantities of carbon dioxide, namely, 1800 and 1700 mg. respectively.   It should be noted that the roots of these plants were removed prior to the determination of the respiratory rate, and also that during the twelve hours previous to this operation, the plants had been maintained at 0° C.   The stimulation resulting from the wounding incident to the removal of the roots, and from the rise in temperature, may therefore be assumed to be, in part at

least, responsible for the fact that these results are considerably higher than those reported by MARTIN for similar material. The latter has determined the respiration of a number of varieties of winter and spring wheat and of rye at still lower temperatures. His results may be summarized as follows:

	Milligrams CO_2 per kilogram of dry weight per hour		
	At 0°	At − 5°	At − 10°
Wheat	182.5–259.3	141.3–188.6	50.3–127.0
Rye	516.9	165.8	70.8

In accordance with the results of GOVOROV, the hardier varieties were found to evolve less carbon dioxide than those less resistant to low temperatures, particularly at − 10°. The relation between wheat and rye at 0° is also similar to that found by GOVOROV.

It should be noted that MARTIN used the same plants for the determination of the respiratory activity at the different temperatures and proceeded from the higher to the lower levels, allowing 12 hours for accommodation to the new temperature. This is important, since there is much evidence that the direction in which the temperature alteration occurs may cause appreciable differences in the results obtained at the new level.

Although the balance of the evidence is in favor of the view that increasing the temperature of plants brings about stimulation of the rate of respiration, there is, however, some contrary evidence. ZIEGENBEIN (17), for example, found no stimulation in respiration on raising the temperature of seedlings of vetch and lupine from 15–20 to 30° C. He used the continuous aspiration method and apparently ample time was allowed for the temperature equilibrum of the tissues with the surrounding atmosphere to become established at the different temperatures.

BLANC (1), also, has concluded that, in the case of etiolated shoots of *Vicia faba*, bean seedlings, and young leaves of rye, the respiratory transition is gradual from one temperature to another and without stimulative effects due either to rising or falling temperature.

Nevertheless, we are confident that, when plant tissue is brought from a lower to a higher temperature for the measurement of its CO_2 production, the resultant curve will typically not assume a level until after a preliminary peak has been passed. We further believe that this peak is largely explainable by the lesser solubility of the CO_2 at the higher temperature; and that the CO_2 of the peak of the curve represents that which was produced by respiration at some time previous to that of the measurement.

Summary and conclusions

1. It has been shown that wound respiration in excised apple twigs is a small and relatively constant fraction of the total respiration.

2. The rate of CO_2 production as measured at any temperature is conditioned by the previous temperature environment of the twigs. If the previous temperature has been higher, a constant level of CO_2 emission is gradually assumed. If the previous temperature has been lower, there is a peak of CO_2 evolution for several hours, after which a level is gradually attained.

3. The lower the previous temperature, the higher is the peak amount of CO_2. This was proved both by twigs kept in storage before measuring the CO_2 output, and by twigs taken directly from the orchard at various temperatures throughout the winter.

4. It is suggested that this temporary excess of CO_2 represents that which was produced during the previous period but which remained dissolved in the twig sap, and which diffuses out of the twigs at the higher temperatures because it then becomes less soluble.

5. Hardier varieties of apples show a lower peak of CO_2 evolution than do the tenderer. In fact, the order of increasing tenderness among 11 varieties coincides almost exactly to that of increasing CO_2 during the peak.

6. Twigs stored at $-2°$ C., $-6°$ C., and $-10°$ C. for periods up to 30 days with and without aspiration of the surrounding air did not show striking changes in chemical composition. However, without aspiration there seems to be slight gain in sugar; and with aspiration there seems to be a slight gain in starch and loss in sugars.

7. It is emphasized that in the measurement of the respiratory rate of any plant tissue the effect of dissolved CO_2, and hence the previous temperature history of the material, must be kept in mind.

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LITERATURE CITED

1. BLANC, L. Recherches expérimentales sur l'influence des variations de température sur la respiration des plantes. Rev. Gén. Bot. **28**: 65–79. 1916.

2. DEHÉRAIN, P., and MAQUENNE, L. Sur la respiration des feuilles à l'obscurité. Acide carbonique reteuu par les feuilles. Compt. Rend. Acad. Sci. Paris. **101**: 887–889. 1885.

3. BEAUMONT, J. H., WILLAMAN, J. J., and DE LONG, W. A. Notes on apparatus for low temperature respiration studies. Plant Physiol. **2**: 487–495. 1927.

4. Govorov, L. I. The diverse characters of winter and spring forms of cereals in connection with the problem of hardiness in winter crops. Bull. Appl. Bot. & Plant Breeding (Trans. title) **13**: 525–559. 1922–3.

5. Johnstone, G. R. Physiological study of two varieties of *Ipomoea batatas*. Bot. Gaz. **80**: 145–167. 1925.

6. Maquenne, L. Sur la respiration des feuilles. Compt. Rend. Acad. Sci. Paris. **119**: 100–102. 1894.

7. Martin, J. H. Comparative studies of winter hardiness in wheat. Jour. Agr. Res. **35**: 493–535. 1927.

8. Müller-Thurgau, H. Über Zuckeranhäufung in Pflanzentheilen in Folge niederer Temperatur. Land. Jahrb. **11**: 751–828. 1882.

9. Olney, A. J. Temperature and respiration of ripening bananas. Bot. Gaz. **82**: 415–426. 1926.

10. Palladin, W. Influence des changements de température sur la respiration des plantes. Rev. Gén. Bot. **11**: 241–257. 1889.

11. Quisumbing, F. A., and Thomas, A. W. Conditions affecting the quantitative determination of reducing sugars by Fehling solution. Elimination of certain errors involved in current methods. Jour. Amer. Chem. Soc. **43**: 1503–1526. 1921.

12. Simon, S. Untersuchungen über das Verhalten einiger Wachstumfunktionen sowie der Atmungstätigkeit der Laubhölzer während der Ruheperiode. Jahrb. wiss. Bot. **43**: 1–48. 1906.

13. Willaman, J. J., and Beaumont, J. H. Preliminary report on the respiration of apple twigs during the winter. Proc. Amer. Soc. Hort. Sci. **1924**: 99–104. 1924.

14. ――――――, and ――――――. The effect of accumulated carbon dioxide on plant respiration. Plant Physiol. **3**: 45–59. 1928.

15. ――――――, and Brown, W. R. The carbon dioxide dissolved in plant sap and its effect on respiration measurements. Plant Physiol. **5**: 535–542. 1930.

16. Zalenski, W. Zur Frage über die Wirkung von Reizen auf die Athmung per Pflanzen. Bot. Centralb. **95**: 251–252. 1904. (Abs. from Russian original.)

17. Ziegenbein, E. Untersuchungen über den Stoffwechsel und die Athmung keimender Kartoffelknollen sowie anderer Pflanzen. Jahrb. wiss. Bot. **25**: 563–606. 1893.

CARBON DIOXIDE DISSOLVED IN PLANT SAP AND ITS EFFECT ON RESPIRATION MEASUREMENTS[1]

J. J. WILLAMAN[2] AND WILLIAM R. BROWN

(WITH THREE FIGURES)

We have undertaken to demonstrate that certain phenomena of "respiration" in plants are not due to CO_2 which is produced during the period of measurement, but are due to the CO_2 which is dissolved in the sap of the tissues. The following paper contains our method for measuring this dissolved CO_2 as well as experiments which lead us to certain conclusions as to its significance.

In the preceding paper (3) it has been shown that storage of apple twigs at a lower temperature, followed by their elevation to a higher temperature, gives rise temporarily to a considerable increase in rate of CO_2 output. Furthermore, the rate at the higher temperature is greater, the lower the previous temperature of storage. The same facts obtain when the twigs are taken directly from the trees: the rate of CO_2 output varies inversely with the orchard temperature at the time of collecting the twigs.

CO_2 has, of course, a solubility in aqueous media that varies inversely with the temperature. Presumably, plant sap as a medium is no exception. This principle can explain the above case. Thus, when twigs are raised to a higher temperature for measurements of CO_2, the temporary flush which is observed indicates not a greater production of CO_2 at that time, but represents CO_2 which was previously produced and which remained dissolved in the tissue sap. After a certain period at the higher temperature a new equilibrium is established and from that time on the output of CO_2 is more or less constant for that temperature.

WILLAMAN and BEAUMONT (11) recently published some results on the effect of allowing CO_2 to accumulate in the atmosphere of a respiration chamber. They state: "When the accumulated CO_2 is removed, the rate of its production immediately assumes a far higher value; and the magnitude of this increased value is possibly proportional to the amount of CO_2 previously accumulated (in the chamber). It is a matter of several hours before the rate assumes a constant value." They introduced some evidence to show that the explanation of this phenomenon lay at least partly in the fact that the dissolved CO_2 increased the hydrion concentration of the tissue and that this stimulated respiration. We believe now that another

part of the explanation lies in the accumulation of CO_2 in the tissues, because of the greater partial pressure of CO_2 in the atmosphere surrounding the twigs. When this partial pressure is reduced, the dissolved CO_2 diffuses out of the twigs and causes the apparent temporary increase in respiratory rate which is always observed. Ultimately, a new equilibrium is established, and the output of CO_2 becomes constant, unless another period of accumulating CO_2 in the chamber intervenes.

The difference in the above two cases is that in the latter the temperature was constant and the CO_2 accumulated in the tissue because it was also accumulating in the atmosphere around the tissue; while in the former case the accumulation in the tissue was only relative because of the difference in solubility of the CO_2 at the different temperatures.

In the preceding paper (3) data are submitted which indicate that those varieties of apples which possessed the greatest winter hardiness seemed to have the lowest rate of CO_2 output, while the tenderer varieties had the highest rate. In the present paper we show that the varietal differences in apparent CO_2 output are paralleled by differences in the amount of dissolved CO_2. The hardier varieties contain less, and the tenderer more, dissolved CO_2.

Although we have only a limited number of determinations of dissolved CO_2 at the present time, they are rather definite in their trend, and it seemed best to publish them simultaneously with those in the preceding paper, because we feel that they partly explain the findings in the latter paper.

In 1885, DEHÉRAIN and MAQUENNE (2) first called attention to the CO_2 dissolved in plant sap and to its possible effect on respiration measurements. Later MAQUENNE (5) attempted to remove this CO_2 by evacuation, but was only partly successful. SIMON (9) has measured the CO_2 production by excised twigs of beech, red oak, basswood and horse chestnut. The twigs were brought from various outdoor temperatures to 22.5° C. for measurement, and SIMON noted that the lower the previous temperature the greater was the production of CO_2 during the early part of the measurement period. This effect was so pronounced that he extended his measurements over a period of seven days in order to avoid that temporary flush. Increasing the temperature has frequently been observed to produce a temporary increase in CO_2 evolution. A very definite effect of this nature was early noted by MÜLLER-THURGAU (6) in potato tubers, and has since been reported for etiolated shoots of *Vicia faba* by PALLADIN, (8), gladiolus bulbs by ZALENSKI, (12), sweet potatoes by JOHNSTONE, (4), and bananas by OLNEY (7).

Apparatus and method

The apparatus as finally used is illustrated in fig. 1, and is largely self-explanatory. The container D can be of glass or of copper. The procedure
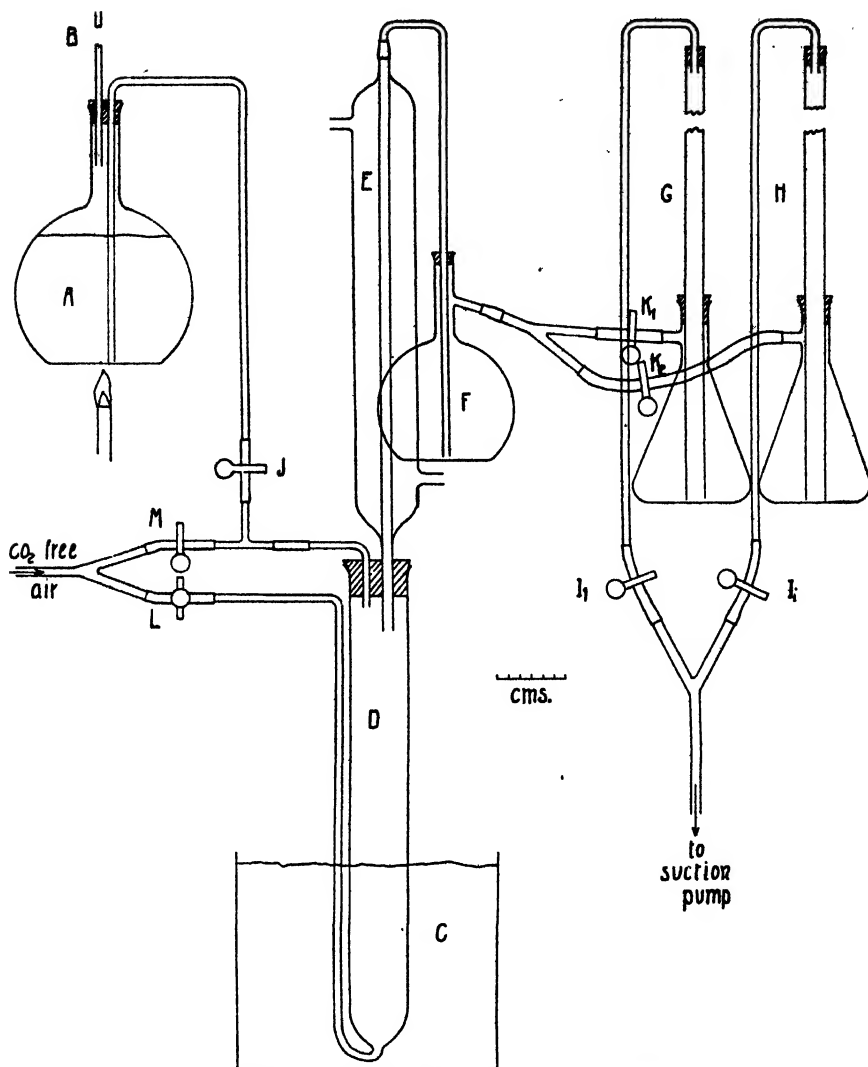
FIG. 1. Diagram of apparatus for determining dissolved CO_2.

is as follows: The bead towers (1) are charged with alkali, usually 25 cc. of 0.2N NaOH, and clamps I_1 and I_2 are closed. About 400 cc. of 95 per cent. alcohol is boiled in A for about 5 minutes to free it of CO_2. The water in bath C is heated to about 90° C. With the apparatus thus in readiness, the sample of twigs, in apples about 150 to 170 gm., is placed in the tube D, with the cut ends up. This must be done at the place where the twigs have been, either in the orchard or in storage, so that the twigs shall not be exposed to a higher or lower temperature until they are in the tube and

stoppered. The small outlet tube is closed with a piece of rubber tubing and a clamp. The tubes are then taken immediately to the laboratory and placed in the apparatus. The pump is started, and clamps J, K_1 and I_1 are opened. J is closed as soon as the twigs are covered with the boiling alcohol. Screw-clamp L is opened enough to allow a slow stream of CO_2-free air to pass through the alcohol. A proper balance will have to be established between the partial vacuum in the apparatus and the temperature of the water bath. For the first 30 or 40 minutes it is desirable to have the temperature of the alcohol as high as possible so as to kill the cells of the twigs. Later it is desirable to have a higher vacuum so as to assist the expulsion of the CO_2 from the tissues. If the alcohol froths too violently, clamp M can be opened momentarily. It is useful to have a manometer connected to the system.

When there is reason to believe that tower G has received the allowable charge of CO_2, tower H is shunted into the circuit. This is done by first opening K_2, then gradually opening I_2 and closing I_1, and finally closing K_1. After one and a half hours, the towers should be changed every 15 min. until it is apparent that all the CO_2 has been obtained. Furthermore, the time required for each material may be determined, and this period arbitrarily used in all subsequent cases without changing the towers. We have found that 120 minutes suffice for apple and cherry twigs.

In titrating, the tower is removed from its flask, the beads allowed to slide into the flask, and the tower rinsed with CO_2-free water. The alkali is then back-titrated with standard acid in the presence of the beads. We prefer the double indicator of Simpson (10), containing cresol red and thymol blue. This gives the CO_2 in one titration and avoids the use of any barium for the precipitation of the carbonate.

Experimental results

A typical determination of dissolved CO_2 by the method described is given in table I. In most cases the exhaustion of the CO_2 was completed in 105 minutes. At the present time we are finding that for most materials we can use a single titration at the end of 120 minutes.

On Feb. 15, 1929, one-year old twigs of Duchess were collected at University Farm and divided into 9 lots. Two were analyzed immediately, 3 were stored at 4–6° C., 2 at 15° C. and 2 at $-22°$ C. They were laid on open shelves in the storage rooms. At intervals they were analyzed for dissolved CO_2. The results are shown in fig. 2.

One immediately obvious fact is that the amount of the contained CO_2 was inversely proportional to the temperature of storage. This is brought out more strikingly when the amounts of CO_2 are plotted against tempera-

TABLE I

REPRESENTATIVE DETERMINATION OF DISSOLVED CO_2
SAMPLE = 92.0 GM. OF DUCHESS TWIGS

TIME	CO_2
minutes	mg.
15	1.89
30	8.45
45	6.93
60	3.15
75	2.39
90	1.38
105	0.88
120	0.13
Total	25.20
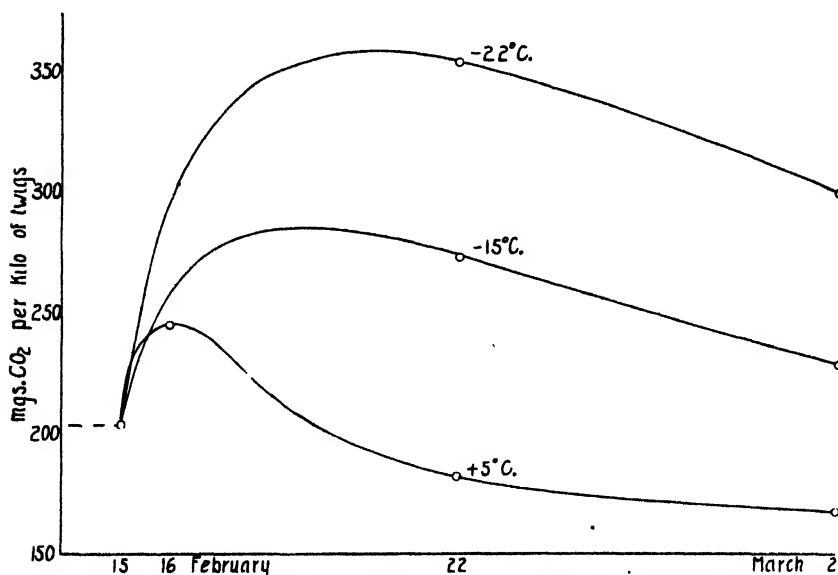
FIG. 2. Amount of dissolved CO_2 in twigs of Duchess stored at various temperatures for various lengths of time.

ture, as in fig. 3. These curves indicate a rapidly increasing solubility of CO_2 in sap at low temperatures.

Fig. 2 also shows that during the period of the experiment the amount of CO_2 at all three temperatures declined. This probably indicates some change in the solution properties of the sap towards CO_2.

On Dec. 28, 1928, samples of twigs of several varieties of apples were collected at the University of Minnesota Fruit Breeding Farm at Excelsior.
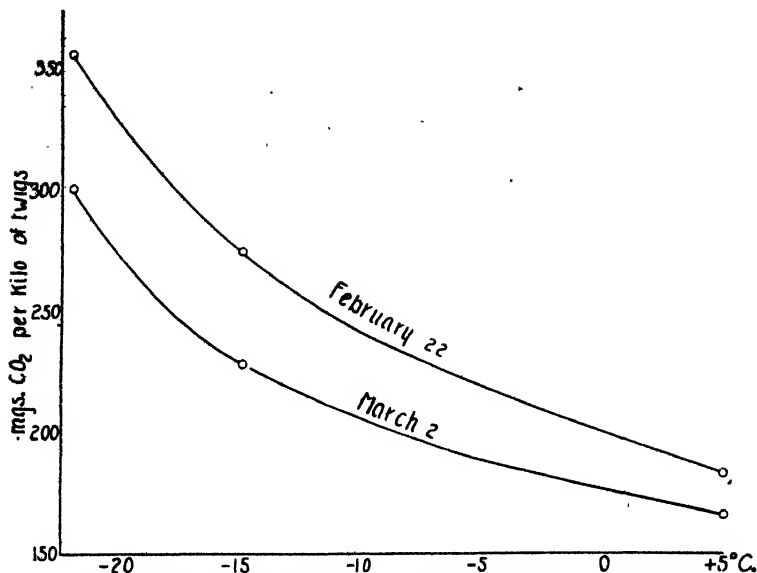
FIG. 3. Same data as in fig. 2, but showing the solubility at various temperatures.

They were stored on open shelves at $-15°$ C. Parts of these samples were used for the respiration measurements recorded in the preceding paper (3). In February some of them were still available; and although they were rather old by this time we decided to measure their dissolved CO_2. The results are given in table II. Although there were only five varieties for which BEAUMONT and HILDRETH have hardiness values, and although they

TABLE II

RELATION OF DISSOLVED CO_2 IN APPLE VARIETIES TO THEIR WINTER HARDINESS

VARIETY	HARDINESS*	CO_2 PER KILO
Duchess	8	202
Patten Greening	11	279
Wealthy	13	315
Wolf River	21	434
Sugar Loaf	25	384
Lyman Perfect	**	276

* According to BEAUMONT and HILDRETH's scale (3, p. 523), the larger the number, the greater is the tenderness.
** Not listed by BEAUMONT and HILDRETH.

had been off the tree for seven weeks, a striking relation is seen between the dissolved CO_2 and the hardiness. Only one variety, Sugar Loaf, is an exception, and this is only one position out of place. In other words, the hardier varieties contain less CO_2 in their sap, and the tenderer contain more.

In the preceding paper it was shown that the hardier varieties give off less CO_2 than the tenderer. Thus the corollary to these facts is that a lower output of CO_2 is accompanied by a lower solvent power for the gas. The explanation for this relation must involve some of the fundamental properties of the twig protoplasm.

Summary and conclusions

A method has been devised for determining the CO_2 dissolved in twig sap. It consists essentially in submerging the twigs in boiling 95 per cent. alcohol in a closed container, and then removing the CO_2 with reduced pressure. The CO_2 is caught in standard alkali and titrated.

The amount of CO_2 contained in apple twigs is of the magnitude of 150 to 250 mg. per kilo at 0° C. The amount varies inversely with the temperature, and may be as high as 360 mg. at $-22°$ C.

We believe that this dissolved CO_2 may explain at least three phenomena of plant "respiration" measurements, meaning by the latter term the measurement of the CO_2 given off by the plant tissue under stated circumstances. These phenomena are as follows:

(1) When apple twigs are raised from a lower to a higher temperature, the output of CO_2 at the higher temperature is temporarily increased above the level that it subsequently attains at that temperature. The effect is greater, the lower the previous temperature. The temporary flush is caused, we believe, by the lower solubility of the CO_2 at the higher temperature.

(2) When plant tissue (twigs, potato tubers, and wheat grain have so far been observed) is stored in a closed chamber and the CO_2 allowed to accumulate, and then the CO_2 swept out and the rate of its output by the tissue measured, this rate is found to rise to a temporary high value. Ultimately, this rate subsides to a lower level characteristic of the temperature used. We believe that this flush may indicate an actual increase in rate of production of CO_2, occasioned by the higher acidity of the sap during the accumulation of CO_2. The peak of CO_2 output is probably also accounted for partly by the diffusion of previously formed CO_2 which was held in solution because of the higher partial pressure of CO_2 in the atmosphere surrounding the tissue.

(3) Those varieties of apples which possess the greatest winter hardiness show the lowest output of CO_2. We have found that they also contain

the lowest amount of dissolved CO_2. What the causal connection is between the two is not known at present.

The generality which we deduce from these results is that in plant respiration studies a careful distinction must be made between the *production* of CO_2 and its *output.* The one may or may not be the same as the other, due to the various factors which affect the amount of dissolved CO_2. The factors known at present are temperature, partial pressure of CO_2 in the atmosphere around the tissue, and the kind of tissue.

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LITERATURE CITED

1. BEAUMONT, J. H., WILLAMAN, J. J., and DE LONG, W. A. Notes on apparatus for low temperature respiration studies. Plant Physiol. **2**: 487–95. 1927.
2. DEHÉRAIN, P., and MAQUENNE, L. Sur la respiration des feuilles à l'obscurité. Acide carbonique retenu par les feuilles. Compt. Rend. Acad. Sci. Paris. **101**: 887–889. 1885.
3. DE LONG, W. A., BEAUMONT, J. H., and WILLAMAN, J. J. Respiration of apple twigs in relation to winter hardiness. Plant Physiol. **5**: 509–534. 1930.
4. JOHNSTONE, G. R. Physiological study of two varieties of *Ipomoea batatus*. Bot. Gaz. **80**: 145–167. 1925.
5. MAQUENNE, L. Sur la respiration des feuilles. Compt. Rend. Acad. Sci. Paris. **119**: 100–102. 1894.
6. MÜLLER-THURGAU, H. Über Zuckeranhäufung in Pflanzentheilen in Folge niederer Temperatur. Land. Jahrb. **11**: 751–828. 1882.
7. OLNEY, A. J. Temperature and respiration of ripening bananas. Bot. Gaz. **82**: 415–426. 1926.
8. PALLADIN, W. Influence des changements de température sur la respiration des plantes. Revue Gén. Bot. **11**: 241–257. 1899.
9. SIMON, S. Untersuchungen über das Verhalten einiger Wachstumfunktionen sowie der Athmungstätigkeit der Laubhölzer während der Ruheperiode. Jahrb. wiss. Bot. **43**: 1–48. 1906.
10. SIMPSON, S. G. A mixed indicator for carbonate-bicarbonate titrations. Jour. Ind. Eng. Chem. **16**: 709–710. 1924.
11. WILLAMAN, J. J., and BEAUMONT, J. H. The effect of accumulated carbon dioxide on plant respiration. Plant Physiol. **3**: 45–59. 1928.
12. ZALENSKI, W. Zur Frage über die Wirkung von Reizen auf die Athmung der Pflanzen. (Abs. from Russian original.) Bot. Centralb. **95**: 251–252. 1904.

INFLUENCE OF LEAFAGE REMOVAL ON ANATOMICAL STRUCTURE OF ROOTS OF *STIPA PULCHRA* AND *BROMUS HORDEACEUS*

KENNETH W. PARKER[1] AND ARTHUR W. SAMPSON[2]

(WITH FOUR FIGURES)

Historical

Numerous investigations have been reported to show the influence of the reduction of chlorophyll-bearing tissue, as in the pruning of fruit trees or in the cutting or grazing of fodder and forage plants, on the subsequent yields. There has been little serious investigation to show the effect of harvesting on the physiological responses of the plant, and more especially on the anatomical structure of the roots.

PRIESTLEY and EVERSHED (8) have shown that root growth is periodic and seasonal in many species remotely related taxonomically. Removal of succulent leafage, however, tends clearly to unbalance, or actually to destroy, any rhythm that might otherwise exist between top and root growth. Moreover, defoliation is definitely correlated with the extent of root growth, such as may be expressed in dry weight or in volume of root expansion. Only a slight reduction in the area of active chlorophyll-bearing parts typically causes a correspondent slowing down in root growth. If the decreased elaboration of organic materials is further intensified by herbage removal, root growth may cease entirely. Defoliation at frequent intervals of the plants studied finally caused destruction of the root system as a whole, and death of the plant. In studies by Sampson (9) plots of *Festuca viridula* in Oregon were harvested three times annually for three successive years. Root and aerial growth, including size of tufts, decreased annually during this treatment.

SAMPSON and MALMSTEN (10) working with many species of bunch grasses and other plants, found that the removal of herbage four to five times in a season, at approximately monthly intervals, resulted in a sharp decline in yield and a marked shortage of life of the vegetation. Cropping two (or in a few vigorous species even three) times in a season, leaving between each harvest an interval of time sufficient for the development of considerable aerial growth, did not jeopardize the forage yield. McCARTY (7) harvesting in Colorado *Agropyron smithii* and *Bulbilis dactyloides* four times in a season, found that growth and the food reserves were so curtailed that the mortality due to winter killing was very high during the

period of diplomacy. Removal of the herbage at such intervals as to result in a reduction of the total aerial dry matter produced was invariably reflected in a decreased root development and in a lesser quantity of food stored in the stem bases and in the roots themselves. Poor development of the root system resulted in the production of an increasingly small quantity of herbage in the following season. The earlier work of ELLETT and CARRIER (3) on blue grass pastures brought out the conclusion that "the total yield of dry matter varies inversely with the number of times the pasture is cut during the growing season." The results of STAPLEDON (12) and of GRABER, NELSON, LEUKEL, and ALBERT (4) are similar. They concluded further that the root systems of grasses are very sensitive to the cutting of the aerial parts.

As previously stated, studies of the anatomy of roots of Gramineae are limited, despite the apparent economic application of such investigations to yield and longevity of this important family of plants. The following texts, however, are somewhat pertinent to the study in question: DE BARY (1), HABERLANDT (5), EAMES and MACDANIELS (2), and WEAVER (13, 14).

JACKSON (6) in the study of barley roots concluded that the root system of a well developed barley plant consists of two forms—a thin branched type, and a thick unbranched type with abundant root hairs. The unbranched root arises from the first node above the grain. Its chief function appears to be that of providing ample water and nutrient salts at the time that vigorous growth is taking place. This function is accomplished by the presence of abundant root hairs and an increased number of large vessels and central ducts, as well as the existence of a stele composed largely of thin-walled elements. Measurements of the cross-sections of the roots, some of which were developed in soil and others in water cultures, were taken in two dimensions at right angles to each other, that of the stele, the ducts, and the entire cross-section of the root. These ratios were obtained by taking as area the product of the two diameters "since the area of an ellipse is proportional to that of its escribed rectangle." The ratio was expressed as $\dfrac{\text{area stele}}{\text{area ducts}}$. Other ratios of area were those of $\dfrac{\text{whole root}}{\text{ducts}}$ and $\dfrac{\text{whole root}}{\text{stele}}$, but these were found to be inconsistent.

Methods of study

THE PLANTS.—Because of the detailed character of the work, only two plant species were used in the experiment. The one was *Stipa pulchra*, a perennial bunch grass which typically occurs in a well developed soil "A" horizon and is recognized as a climax species in the true savanna of the Sonoran life zone of California; the other was *Bromus hordeaceus*, an an-

nual of Mediterranean origin now found extensively in the Sonoran zones. Both species are of great value as pasture plants.

THE CULTURES.—In order to facilitate critical study and measurement of the roots and to eliminate the variables of soil and sand, a water culture, formulated by Professor D. R. HOAGLAND, was used. The culture solution, in addition to the regular constituents, contained in the formula a small quantity of so-called "A-Z" solution, which served to offset any deficiency due to the need of rare elements. The constitution of the stock and culture solutions is shown in table I.

TABLE I

SALTS	MOLAR CONCENTRA- TION, PER LITER STOCK SOLUTION	MOL SOLUTION PER LITER	IONS	
	gm.	*cc.*	*ppm.*	
$Ca(NO_3)_2 \cdot 4H_2O$	236.2	3.9	Ca	157
KNO_3	101.1	3.6	NO_3	709
			K	181
$MgSO_4 \cdot 7H_2O$	246.5	2.2	Mg	55
			SO_4	217
KH_2PO_4	136.2	1.1	PO_4	105

The culture required 2 cubic centimeters of a 0.5 per cent. solution of ferric tartrate per liter of solution, to supply iron.

THE TISSUES.—Root tips were taken from adventitious roots of the two species studied when the plants had been grown for 105 days in the water cultures. During this period of growth one set of plants had been harvested by cutting within one-half inch of the stem base on seven occasions at 15-day intervals; the other set of plants had not been harvested or otherwise treated during the period of growth. The root segments were cut at 2.5 cm. from the growing point.

FIXATION.—Part of the material was fixed with Allen's solution; the other part with alcohol-formalin-acetic no. 1 solution for a period of 70 hours. Allen's solution was prepared as follows:

Chromic acid .... 1 gram
Acetic acid .... 1 gram
Urea .... 0.5 gram
Distilled water .... 100 cc.

The material was then washed in running water for 12 hours after fixing.

The alcohol-formalin acetic no. 1 solution was prepared as follows:

50 per cent. alcohol .... 100 cc.
Formalin .... 6.5 cc.
Acetic acid .... 2.5 cc.

No washing was necessary following fixing.

EMBEDDING.—Following fixation the material was dehydrated by carrying it through a graduated series of increasing alcoholic concentrations to absolute alcohol. The material was then infiltrated with a paraffin solvent, the alcohol being displaced by benzol. The material was then infiltrated with paraffin by adding paraffin shavings to the benzol which contained the plant material. The vials containing this material were kept in a sparkless paraffin oven for 24 hours. Following this the paraffin-benzol solution was decanted and melted paraffin was added. The paraffin was changed twice during the 24-hour period, following which the material was allowed to remain 48 hours in the oven.

The material was then poured into pasteboard boxes and cooled with waste alcohol. The blocks were sectioned with a rotary microtome in thicknesses of 7 microns and 15 microns, respectively, and were mounted on glass slides in the usual manner. The slides were passed through xylol, alcohol, and water to remove paraffin and to infiltrate the section with the water to facilitate staining. A modification of Haidenhain's iron-alum haematoxylin stain was used.

MEASUREMENTS.—Measurements were made with a Filar micrometer which had been calibrated with a stage micrometer. Two diameters of the cross-section of the whole root, the stele, and the ducts were obtained.

Results and discussion

There was a marked difference in the size of the roots of the plants harvested compared with those not harvested. In *Stipa pulchra* the average diameter of the cross-section of the whole root was 0.827 mm. in the plants not harvested and 0.517 mm. in those harvested seven times at 15-day intervals, or a difference of 37.5 per cent. (table II, figs. 1 and 2). The area of the cross-section of the whole root was 0.684 sq. mm. in the plants not harvested and 0.267 sq. mm. in those harvested, or a difference of 61.0 per cent. The diameter of the stele was 0.367 mm. in the plants not harvested and 0.227 mm. in those harvested, or a difference of 38.2 per cent. The area of the stele was 0.135 sq. mm. in the plants not harvested and 0.052 sq. mm. in those harvested, or a difference of 61.5 per cent. The number of ducts averaged 9 in the untreated plants and 5 in those harvested, a difference of 44.5 per cent. The total area of the ducts was 0.009 sq. mm. in the plants not harvested and 0.004 sq. mm. in those harvested, or a difference of 55.5 per cent.
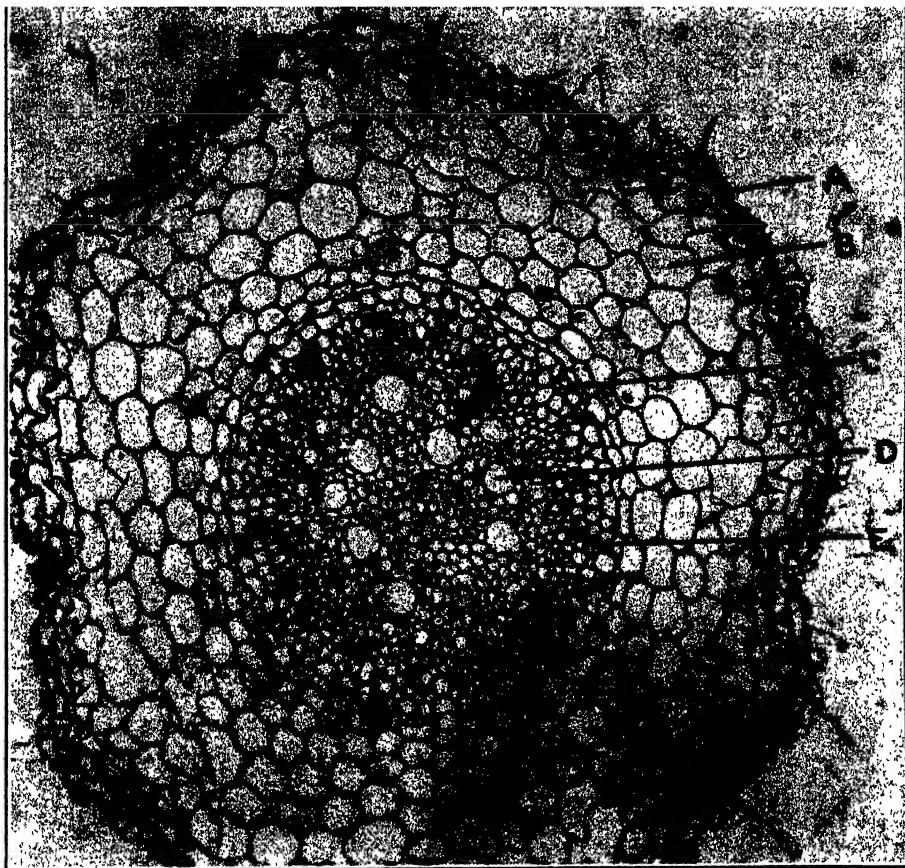
FIG. 1. Cross-section of root of *Stipa pulchra,* not harvested.   143 ×.

In *Bromus hordeaceus* similar results, although somewhat less marked, were obtained in the roots of harvested and of unharvested plants (table II, figs. 3 and 4). Here again, the harvested plants were clipped at 15-day intervals over a period of 105 days. The dates of cutting were the same as for *Stipa pulchra.*

In the plants of *Bromus* not harvested the diameter of the whole root was 0.606 mm., as compared with 0.434 mm. in those harvested, with a difference of 28.4 per cent. The area of the whole root was 0.367 mm. in the untreated plants as compared with 0.189 mm. in those harvested, a difference of 48.5 per cent. The diameter of the stele was 0.237 mm. in the control plants, as compared with 0.181 mm. in those harvested, with a difference of 23.7 per cent. The area of the stele was 0.056 sq. mm. in the untreated, as compared with 0.033 sq. mm. in the harvested plants, with a
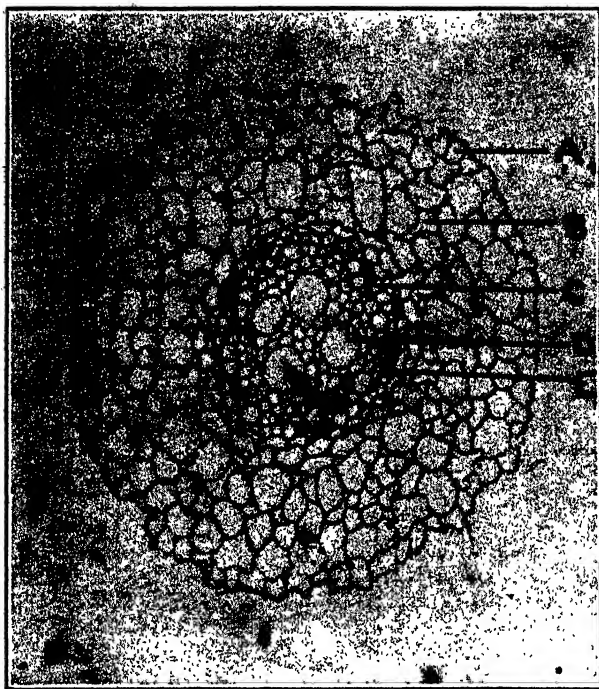
FIG. 2. Cross-section of root of *Stipa pulchra*, harvested frequently. 143 ×
A, epidermis; B, cortex; C, endodermis; D, vessel; E, pericycle.

difference of 41.1 per cent. The number of ducts averaged 6 in the un-treated plants, as compared with 2 in the harvested plants, with a difference of 66.6 per cent. The total area of the ducts was 0.008 sq. mm. in the untreated plants, as compared with 0.003 sq. mm. in those harvested, with a difference of 62.5 per cent.

The marked difference in the size of the roots in the *Bromus* plants harvested as compared with those untreated is probably accounted for by (1) the decreased elaborated nutrition of the harvested plants as a whole, (2) the decreased absorptive capacity of the roots, and (3) a correspond-ing decreased transpiration stream, all of which results from the frequent reduction in leaf area. Regeneration of the aerial growth was strongly stimulated by harvesting the tops. This likewise resulted in an appreciable prolongation of the growth cycle of the herbage. This vigorous regenera-tion growth accounts for an unequal utilization of the elaborated foods, resulting in weak root growth as a whole and a corresponding decrease in the size and the differentiation of the root cross-section. The presence of a larger number of ducts in the untreated plants would appear to be asso-ciated with their relatively larger requirements for water and mineral

TABLE II

COMPARATIVE ANATOMY OF PLANTS HARVESTED FREQUENTLY AND THOSE NOT HARVESTED

	Stipa pulchra CONTROL	Stipa pulchra HARVESTED	DIFFERENCE Stipa pulchra	B. hordeaceus CONTROL	B. hordeaceus HARVESTED	DIFFERENCE B. hordeaceus
			per cent.			per cent.
Diameter whole root in mm..........	0.827	0.517	37.5	0.606	0.434	28.4
Area of whole root in sq. mm......	0.684	0.267	61.0	0.367	0.189	48.5
Diameter of stele in mm..........	0.367	0.227	38.2	0.237	0.181	23.6
Area of stele in sq. mm..........	0.135	0.052	61.5	0.056	0.033	41.1
Number of ducts	9	5	44.5	6	2	66.6
Area of ducts in sq. mm..........	0.009	0.004	55.5	0.008	0.003	62.5
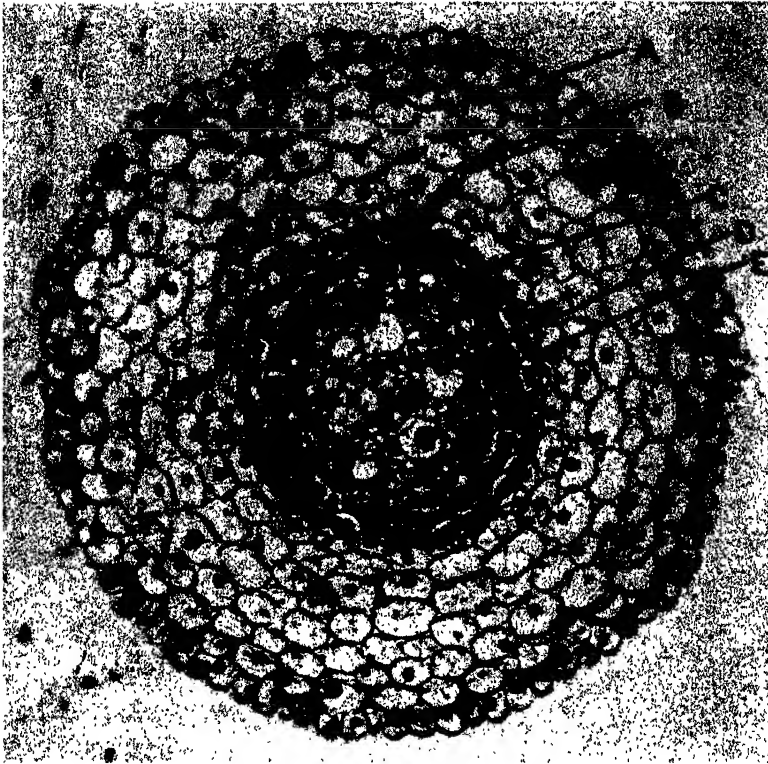
FIG. 3. Cross-section of root of *Bromus hordeaceus*, not harvested. 143 ×.

salts to compensate for their greater size and the balance in their top-root ratio.

An interesting contrast was noted in the production of root hairs. The roots of *Stipa pulchra* grown in water culture produced root hairs which appeared a short distance behind the meristematic region of the root tip. These organs, however, died off as newer ones were formed nearer the root tip. In contrast, the roots of seedlings of *Bromus hordeaceus* produced hairs, but as the plants became older the roots were devoid of such growth and were quite smooth. Possibly this behavior was due in part to adaptation of the plant to the oxygen supply of the solution; the fact that the more elaborate root system was more exposed to the air by the lowering of the solution in the jars; or because sufficient gas was supplied by the more elaborate aerial parts, as observed by SNOW (7). In neither *Stipa pulchra* nor *Bromus hordeaceus*, however, did the frequently clipped plants, after the first month of growth, produce root hairs.

FIG. 4. Cross-section of root of *Bromus hordeaceus*, harvested frequently. 143 x.
A, epidermis; B, cortex; C, endodermis; D, vessel; E, pericycle.

Conclusions

1. Water cultures as here used have proven of great value in the study of root behavior and anatomical differentiation of the species employed.

2. Frequent removal of the aerial growth in *Stipa pulchra* resulted in a poorly developed root structure, in that the diameter of the whole root, the diameter of the stele, and the number of ducts were invariably smaller than in roots of the same age of untreated plants.

3. Frequent removal of the aerial growth of *Bromus hordeaceus* also resulted in a poorly developed root structure, in that the diameter of the whole root, the diameter of the stele, and the number of ducts were smaller than in roots of the same age of untreated specimens.

4. In the cultures where the leaf blades of *Stipa pulchra* were not harvested, root hairs were produced. In contrast, the roots of unharvested plants of *Bromus hordeaceus*, although producing root hairs when very young were later devoid of such structures. This disappearance of the root hairs might be accounted for by the presumed accommodation of the species to the oxygen supply.

5. Where the succulent aerial growth of *Stipa pulchra* and *Bromus hordeaceus* was frequently removed by clipping, the roots were devoid of hairs.

6. This experiment supports the more general observations of similar leafage removal studies with many species during the period of active growth under field conditions. Where it is important to maintain the herbaceous cover, as for pasturage, or where an elaborate, robust root system is necessary to bind the soil in order to control erosion, frequent leafage removal should be avoided.

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LITERATURE CITED

1. DE BARY, A. Comparative anatomy of the vegetative organs of the phanerogams and ferns. Oxford, Clarendon Press. 1884.
2. EAMES, A. J. and MacDANIELS, L. H. Introduction to plant anatomy. McGraw-Hill Co., New York. 1925.
3. ELLETT, W. B. and CARRIER, LYMAN. The effect of frequent clipping on total yield and composition of grasses. Jour. Amer. Soc. Agron. 7: 85–87. 1915.
4. GRABER, L. F., NELSON, N. T., LEUKEL, W. A., and ALBERT, W. B. Organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants. Wis. Agr. Exp. Sta. Res. Bull. 80. 1–128. 1927.
5. HABERLANDT, G. Physiological plant anatomy. Macmillan Co., New York. 1914.
6. JACKSON, VIOLET G. Anatomical structure of the roots of barley. Ann. Bot. 36: 21–39. 1922.
7. McCARTY, EDWARD C. Grazing intensities and food relations in *Agropyron smithii*, (thesis, University of California, 1927, in press).
8. PRIESTLEY, J. H. and EVERSHED, A. F. C. H. Growth studies, I. A quantitative study of the growth of roots. Ann. Bot. 36: 225–237. 1922.
9. SAMPSON, ARTHUR W. Natural revegetation of range lands based upon growth requirements and life history of the vegetation. Jour. Agr. Res. 3: 93–147. 1914.
10. ———— and MALMSTEN, H. E. Grazing periods and forage production on the national forests. U. S. Dept. Agr. Bull. 1405. 1926.

11. SNOW, L. M.   The development of root hairs.   Bot. Gaz. 40: 12–48. 1905.

12. STAPLEDON, R. G.   The seasonal productivity of herbage grasses with particular reference to the influence of different systems of cutting on indigenous and non-indigenous strains, respectively. Welsh Plant Breeding Sta., series H. no. 3, season 1920–1923. 1924.

13. WEAVER, J. E.   Root development of field crops.   McGraw-Hill Co., New York.   1926.

14. ——————.   Root development of vegetable crops.   McGraw-Hill Co., New York.   1927.

DISTRIBUTION OF MOISTURE, DRY MATTER, AND SUGARS IN THE MATURING CORN STEM[1]

F. A. WELTON, V. H. MORRIS, AND A. J. HARTZLER[2]

(WITH SIX FIGURES)

Introduction

A series of physiologic studies of corn has been undertaken cooperatively by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. D. A., and the Dept. of Agronomy, Ohio Agricultural Experiment Station. Among the studies inaugurated, one related to the distribution of moisture, dry matter and sugars in the stem during the period from tasseling to maturity. The results obtained in the study are presented in this paper.

Material and methods

The investigation has been carried on for two years. There was used in 1927 the variety Clarage, a yellow dent corn which matures about midseason at Wooster, and in 1928 Burr Leaming, a double cross of four selfed lines of dent corn, late maturing. The corn was grown on a Wooster silt loam which is in a good state of fertility.

The first samples were taken as soon as the plants were fully tasseled and the sampling was continued at weekly intervals to maturity. The samples consisted of 6 plants each having 12 nodes. The stalks were cut off at the surface of the soil. The leaves, including the sheaths, the tassels, and the ears were all discarded. The stems were then divided into segments, the division in each case being made at the growing point just above the node. The term "segment" as used in this paper refers to a section of stem which consists of an internode and the node above. Each stem, therefore, was divided into 12 segments which were numbered consecutively, the lowest being designated always as "1". The corresponding segments of the 6 plants were grouped together to form composite samples representing the different segments. For each date of sampling, therefore, a series of 12 composite samples was obtained.

The segments were cut into small pieces and aliquot samples preserved in boiling alcohol. Later these samples were placed in Soxhlet extractors and extracted for 8 hours with 80 per cent. alcohol. The following determinations were made: total dry matter, reducing sugars, and sucrose. An attempt was made to determine starch, but there was not enough present to give a test by the usual quantitative method.

[1] Contribution from the Department of Agronomy, Ohio Agr. Experiment Station.
[2] Associate, Associate and former Assistant in Agronomy respectively.

The data are expressed on the fresh weight basis. The percentage of "residual dry matter" reported is the total dry matter minus the total sugars. MASON and MASKELL (1) have suggested the use of this method of expression on the basis that since the major fluctuations in dry weight of relatively mature plant tissues are due to variations in carbohydrates, it may be assumed that the remaining fraction of the dry weight will be relatively constant.

Results

COMPOSITION OF THE STEM BY SEGMENTS

The percentage composition of the successive segments of the stem as found in the variety Clarage in 1927 is given in table I and presented

TABLE I

COMPOSITION OF SUCCESSIVE SEGMENTS OF THE CORN STEM
VARIETY—CLARAGE, 1927

SEGMENTS	RESIDUAL DRY MATTER	MOISTURE	FREE REDUCING SUGARS	SUCROSE
	per cent.	per cent.	per cent.	per cent.
1	11.8	84.6	1.2	2.4
2	11.3	84.7	1.4	2.6
3	10.7	84.5	1.7	3.1
4	11.1	83.5	1.9	3.5
5	11.5	82.8	1.9	3.8
6	12.1	81.7	2.0	4.2
7	12.8	80.7	2.1	4.4
8	13.1	80.2	2.2	4.4
9	12.3	80.6	2.5	4.6
10	13.5	80.7	2.2	3.6
11	12.6	81.5	2.2	3.7
12	14.7	79.5	2.2	3.6

graphically in fig. 1. The data are the averages of the eight series of samples taken from tasseling to maturity.

The percentage of residual dry matter increased from 11.8 per cent. in the lower part of the stem to 14.7 per cent. in the upper part. The percentage of moisture, on the other hand, decreased from 84.6 per cent. in the lower part of the stem to 79.6 per cent. in the upper part. The percentage of reducing sugars and sucrose increased from 1.2 per cent. and 2.4 per cent., respectively, in the lower part of the stem to 2.5 per cent. and 4.6 per cent. in the 9th segment, and then decreased slightly.

The percentage composition of Burr Leaming for 1928 is given in table II and presented graphically in fig. 2. These figures represent the averages
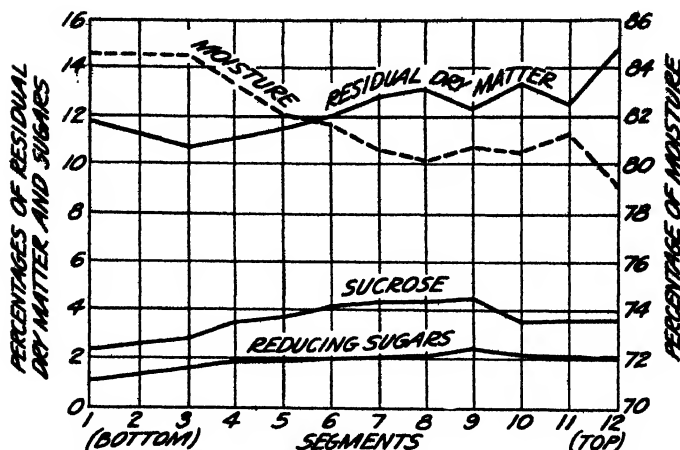
FIG. 1.   Percentage composition of successive segments of the corn stem.   Average of
eight samples taken from tasseling to maturity.
Variety—Clarage, 1927.

TABLE II

COMPOSITION OF SUCCESSIVE SEGMENTS OF THE CORN STEM
VARIETY—BURR LEAMING, 1928

SEGMENTS	RESIDUAL DRY MATTER	MOISTURE	FREE REDUCING SUGARS	SUCROSE
	per cent.	per cent.	per cent.	per cent.
1	11.9	80.5	3.5	4.1
2	12.2	80.4	3.5	3.9
3	13.1	79.1	3.2	4.6
4	13.3	78.6	2.9	5.2
5	14.4	77.4	2.8	5.4
6	14.9	77.0	2.6	5.5
7	16.1	75.9	2.5	5.5
8	16.4	75.4	2.4	5.8
9	16.0	75.8	2.4	5.8
10	16.0	75.7	2.4	5.9
11	15.7	75.9	2.4	6.0
12	17.1	74.6	2.5	5.8

of the six series of samples taken from tasseling to maturity.   In general,
the composition of the successive segments of Burr Leaming stems showed
the same trend as did that of Clarage.   The residual dry matter increased
from 11.9 per cent. in the lower part of the stem to 17.1 per cent. in the
upper part.   The moisture content varied from 80.5 per cent. in the lower
part of the stem to 74.6 per cent. in the upper part.   Reducing sugars de-
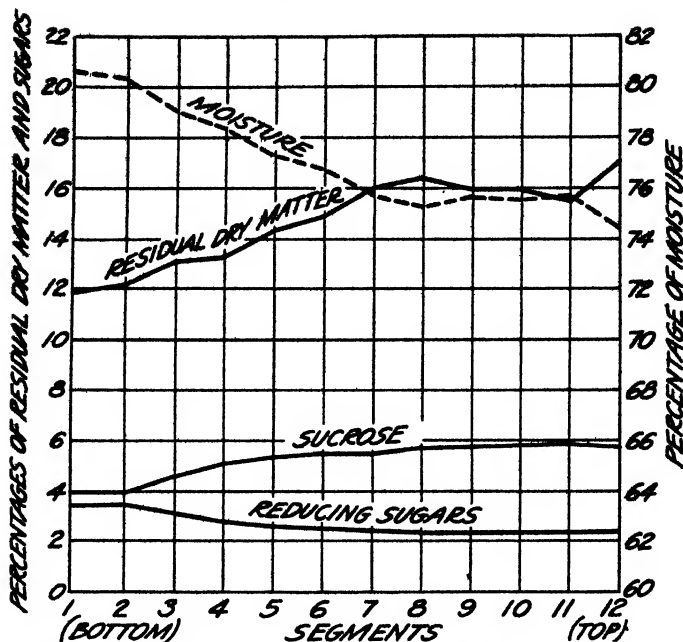
FIG. 2. Percentage composition of successive segments of the corn stem. Average of six samples taken from tasseling to maturity. Variety—Burr Leaming, 1928.

creased from 3.5 per cent. in the lower part of the stem to 2.5 per cent. in the upper, while sucrose increased from 4.1 per cent. in the lower to 5.8 per cent. in the upper part.

SEASONAL CHANGES

The data so far show the gradients of residual dry matter, moisture, and sugars as they occurred between different parts of the corn stem, considering the period between tasseling and maturity as a whole. In order to determine the seasonal changes which took place in the stem, the analyses of the 12 segments for each sampling date were averaged, thus giving the average composition of the stem as a whole for each date.

The percentage composition at different periods from tasseling to maturity for the variety Clarage is given in table III and presented graphically in fig 3. There was a rather rapid increase in residual dry matter from 7.9 per cent. to 12.9 per cent. during the early part of the period, followed by a more gradual increase. There was a correspondingly rapid decrease in moisture content during the early period from 87.1 per cent. to 79.5 per cent. but during the last two weeks the moisture content actually increased, due partly to weather conditions and partly perhaps to

TABLE III

COMPOSITION OF ENTIRE CORN STEM AT DIFFERENT PERIODS FROM TASSELING TO MATURITY
VARIETY—CLARAGE, 1927

DATE	RESIDUAL DRY MATTER	MOISTURE	FREE REDUCING SUGARS	SUCROSE
	per cent.	per cent.	per cent.	per cent.
August 2	7.9	87.1	2.4	2.6
" 9	11.2	84.4	2.5	1.9
" 16	12.1	82.8	2.5	2.6
" 23	12.9	80.9	1.9	4.3
" 29	13.0	79.5	2.1	5.4
Sept. 7	13.6	79.5	1.5	5.4
" 14	13.7	80.0	1.7	4.6
" 20	14.2	82.5	1.0	2.3
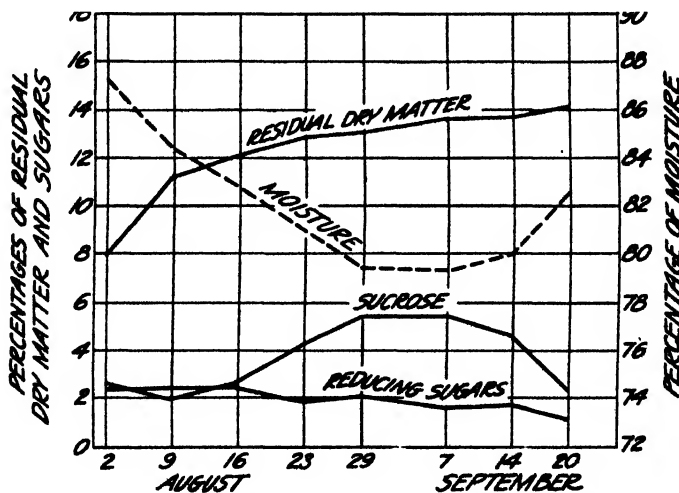
FIG. 3.   Percentage composition of entire corn stem at different periods from tasseling to maturity.   Variety—Clarage, 1927.

the rapid loss of sugars.   The quantity of reducing sugars fluctuated considerably but after the middle of August it trended quite definitely downward.   In general the quantity of sucrose increased until the end of the first week in September at which time the ear was well developed, but after that date it declined gradually for a brief time and then more rapidly.

The average composition of the stem of Burr Leaming at successive periods from tasseling to maturity is given in table IV and presented graphically in fig. 4.   Residual dry matter increased from 13.9 to 17.4 per

TABLE IV

COMPOSITION OF ENTIRE CORN STEM AT DIFFERENT PERIODS FROM TASSELING TO MATURITY
VARIETY—BURR LEAMING, 1928

DATE	RESIDUAL DRY MATTER	MOISTURE	FREE REDUCING SUGARS	SUCROSE
	per cent.	per cent.	per cent.	per cent.
August 10	13.9	80.0	3.1	3.0
" 16	14.0	78.7	3.3	4.0
" 23	14.1	77.9	2.6	5.4
" 30	14.8	76.9	2.7	5.6
Sept. 6	14.5	76.5	2.4	6.6
" 14	17.4	73.0	2.2	7.4
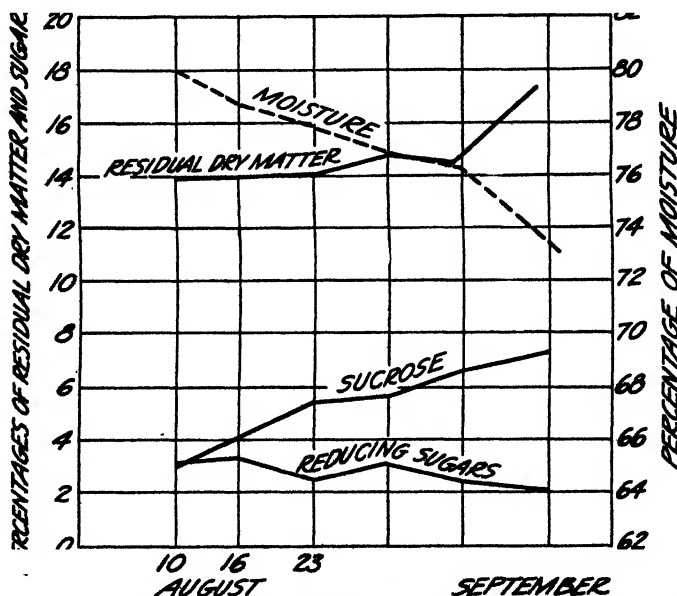
FIG. 4. Percentage composition of entire corn stem at different periods from tasseling
to maturity. Variety—Burr Leaming, 1928.

cent. while moisture decreased from 80.0 to 73.0 per cent.   On Aug. 16 the
reducing sugars were highest, 3.3 per cent. and then they decreased to 2.2
per cent. on Sept. 14; while sucrose increased gradually throughout the
period of sampling, making a total gain of 4.4 per cent.

TOTAL AMOUNT PER SEGMENT

Since there is considerable variation in the size of the segments from
different parts of the stem as is shown by the green weight and length of

each segment it seemed of interest to calculate the quantity of the various constituents per segment. The results of such calculation for the variety Clarage are given in table V and presented graphically in fig. 5. These

TABLE V

TOTAL QUANTITY OF CONSTITUENTS IN EACH SEGMENT
VARIETY—CLARAGE, 1927

SEGMENT	RESIDUAL DRY MATTER	MOISTURE	FREE REDUCING SUGARS	SUCROSE	GREEN WEIGHT OF SEGMENT	LENGTH OF SEGMENT
	gm.	gm.	gm.	gm.	gm.	inches
1	6.8	48.6	0.7	1.4	57.5	4.5
2	10.3	76.7	1.3	2.4	90.7	6.8
3	10.3	82.1	1.6	3.0	97.0	8.1
4	9.7	73.2	1.7	3.1	87.7	8.7
5	8.4	61.0	1.4	2.9	73.7	8.9
6	7.2	48.6	1.2	2.5	59.5	9.2
7	5.8	36.2	1.0	2.0	45.0	8.6
8	4.1	25.6	0.7	1.4	31.8	7.9
9	2.9	19.1	0.5	1.1	23.6	7.9
10	2.3	14.2	0.4	0.6	17.5	7.5
11	1.7	10.6	0.3	0.5	13.1	7.2
12	1.3	8.0	0.2	0.3	9.8	7.1
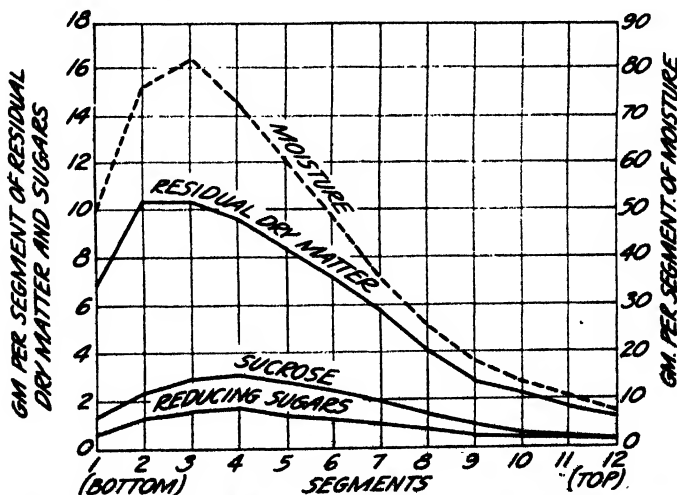
FIG. 5. Total amount in each segment. Average of the eight samples taken from tasseling to maturity. Variety—Clarage, 1927.

data, as mentioned before, represent in each case the average of the series of samples taken from tasseling to maturity.

The total dry matter was a little lower in the first than in the second and third segments, which were equal, but after that it decreased regularly, the twelfth segment containing 9 grams, or 87.4 per cent. less than the second and third, the heaviest two. The moisture content was highest, 82.1 grams, in the third segment—from there it decreased regularly in both directions, reaching a minimum in the twelfth which contained only 8 grams, a decrease of 90.3 per cent.

Residual dry matter decreased from 10.3 grams in the second segment to 1.3 grams in the twelfth. Moisture decreased from 82 grams in the third segment to 8 grams in the twelfth. Reducing sugars and sucrose were highest in the fourth segment, and decreased from 1.7 and 3.1 grams, respectively, to 0.2 and 0.3 gram in the twelfth.

The amounts of the various constituents in each segment of the variety Burr Leaming are given in table VI and presented graphically in fig. 6.

TABLE VI

TOTAL AMOUNT OF CONSTITUENTS IN EACH SEGMENT

VARIETY—BURR LEAMING, 1928

SEGMENT	RESIDUAL DRY MATTER	MOISTURE	FREE REDUCING SUGARS	SUCROSE	GREEN WEIGHT OF SEGMENT	LENGTH OF SEGMENT
	gm.	gm.	gm.	gm.	gm.	inches
1	9.8	62.8	2.7	3.1	78.4	4.8
2	11.4	71.5	3.1	3.5	89.5	6.2
3	11.8	67.6	2.8	4.0	86.2	7.6
4	10.5	58.0	2.2	3.9	74.6	7.9
5	9.2	46.9	1.7	3.3	61.1	8.1
6	7.6	36.8	1.3	2.7	48.4	8.0
7	5.8	26.3	0.9	1.9	34.9	7.3
8	4.1	18.6	0.6	1.4	24.7	7.0
9	3.2	14.3	0.5	1.1	19.1	7.0
10	2.5	11.1	0.4	0.9	14.9	6.8
11	1.9	8.5	0.3	0.7	11.4	6.8
12	1.4	5.8	0.2	0.5	7.9	7.0

The residual dry matter increased from the bottom, reaching a maximum of 11.8 grams in the third segment, from which point it decreased gradually to the top, or twelfth segment, which contained 1.4 grams, or 88.1 per cent. less than the third segment. Beginning at the bottom the moisture content reached a maximum in the second segment from which point it decreased gradually to the top, the twelfth segment containing 91.9 per cent. less than the second. The reducing sugars reached a maximum in the second
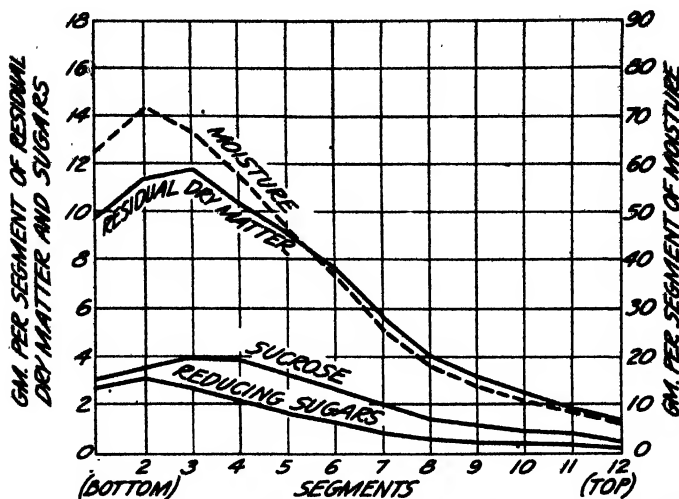
FIG. 6.   Total amount in each segment.   Average of the six samples taken from tasseling to maturity.   Variety—Burr Leaming, 1928.

segment and from there decreased gradually to the twelfth which contained only 0.2 gram, or 93.6 per cent. less than the second.   The quantity of sucrose reached a maximum in the third segment from which point it decreased gradually to the twelfth which contained 0.5 gram or only 87.5 per cent. as much as the third or highest.

These results indicate that as far as the relative amounts of residual dry matter, moisture, and sugars are concerned, the maximum was reached in the second, third, or fourth segment and the amounts in the remaining segments decreased rapidly toward the upper part of the plant.   Calculation shows that more than 50 per cent. of the total amounts of these constituents in the stem were in the lowest four segments.

Summary

The data obtained in this study of the distribution of residual dry matter, moisture, and sugars in the maturing corn stem indicate the following general conclusions:

The percentages of residual dry matter and sucrose were in general larger in the upper part of the corn stem than in the lower part.   The percentage of moisture was larger in the lower part of the stem.

The percentage of residual dry matter increased and the percentage of reducing sugars decreased progressively from the tasseling stage to maturity in both varieties.   The percentage of sucrose increased and the percentage of moisture decreased in the Clarage variety in the early part of the season, but the trend of each was reversed at maturity; the per-

centage of sucrose increased and the percentage of moisture decreased in the Burr Leaming throughout the period studied, thus reversing the trend of these two constituents in the Clarage variety in the latter part of the season. This apparent inconsistency may have been due to immaturity of the Burr Leaming.

The total amount of residual dry matter, moisture, and sugars per segment reached a maximum in the region of the second, third, or fourth segment and decreased rapidly toward the upper part of the plant. The lowest four segments, which constitute about 0.25 of the total length of the stem, contained over 50 per cent. of the total amounts of these constituents in the stem.

LITERATURE CITED

1. MASON, T. G., and MASKELL, E. J. Studies on the transport of carbohydrates in the cotton plant. Memoirs of the Cotton Research Station. Trinidad. 1928.

EFFECT OF MOLDS ON TEMPERATURE OF STORED GRAIN[1]

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(WITH TWO FIGURES)

Although the presence of fungi on stored grain has been known for a long time, the relation of their growth to bin-burning is imperfectly understood. In recent years, however, because of the changes in the methods of handling, much grain has been stored in such condition that molding and bin-burning resulted. Common observation showed that the molding and heating occurred approximately at the same time, indicating a possible interdependence of one upon the other. Grain in elevators, in farmers' granaries, and in transit, all were similarly affected with a correspondingly great loss. The problem, therefore, was a broad one, necessitating the consideration of the relationship between the molds found on bin-burned grain and the production of "heating," the environmental factors favorable to mold growth, and the effects produced.

It is undoubtedly true that temperatures produced in the bin are due to the interaction of several factors. These include the heat of germination itself and temperatures generated by the growth of molds developing on the grain saprophytically. The present investigation was undertaken with the object of isolating and evaluating the rôle of each of these two sources of heat in the temperature increases occurring in stored grains.

The fact that molds may generate high temperatures when grown on suitable organic substances has often been shown in the past. Mention only need be made of the work of COHN (4) on barley, MORGENTHALER (14) on wheat, rye and speltz, McHARGUE (12) and THOM and LE FEVRE (19) on corn meal, KÖNIG (10) with cotton, and MIEHE (13) on hay to indicate the numerous workers and the wide range of materials on which this phenomenon has been observed. However, there seems to have been some idea that living seeds would not serve as a suitable medium for mold growth.

Thus, BAILEY (3) and his coworkers as well as earlier investigators, QUAM (17), DUVEL (8) and BABCOCK (2), who were studying the effect of moisture content of seeds upon respiration, seem to have overlooked the fact that unless special care was taken, the same point at which mold growth began was also the point at which they observed marked increases in CO_2 production. PEIRCE (16) and his associates (7), on the other hand, in their studies on the amount of heat liberated by germinating seeds, pointed out that there was no marked rise in temperature in their flasks except where microorganisms were present. JAMES, RETTGER and THOM (9) also, showed that molds as well as bacteria could produce thermogenesis in grain. In one experiment with sterilized corn they produced a temperature of 51° C. with *Aspergillus fumigatus* Fres.

The problem thus became a study of the cause of thermogenesis in the case of oats, barley and wheat by making a comparison of the temperatures caused by the respiration of the germinating grain and those due to fungous growth.

Materials and methods

The seeds used were produced from the Agronomy farm at Iowa State College and with the exception of the barley were all of the crop of the current year. Because of an epidemic of barley scab during that season, seeds from the previous year which were free from scab were substituted as more desirable.

The seeds were divided into 200-gram samples placed in cheese-cloth bags for convenience in handling during sterilization. The bags were removed when the sample was placed in the Dewar flask for observation of temperature rise. In observing the temperature of germination the seeds were sterilized either by submerging them in an alcohol solution of mercuric chloride or by dry heat. The mercuric chloride solution was made up according to the formula used by NABOKICH (15) : 15 grams of mercuric chloride, 500 grams of 95 per cent. ethyl alcohol and 3,500 grams of water. After mixing, the solution was allowed to stand over night before being used. The samples were immersed in this solution for 15 minutes and then washed in six changes of sterile distilled water by decantation. Following this treatment the seeds were placed in sterile water for twelve hours before being transferred to the flasks. The dry heat sterilization was carried out according to the method described by ATANASOFF and JOHNSON (1). The grain was thoroughly dried and then held at a temperature of 100° C. for thirty hours. The dry heat method was successful with wheat and oats, but the barley was killed by the treatment and, therefore, that phase of the problem was omitted. After cooling, the seed was covered with sterile water for twenty-four hours and then transferred to sterile Dewar flasks under aseptic conditions.

The chambers used to measure the temperatures of germination and mold growth were commercial thermos bottles, plugged with sterile cotton. Thermometers were inserted through the cotton to record the temperatures. Bottles and thermometers were sterilized with the alcoholic mercuric chloride solution and rinsed in sterile water before using. Readings were made at twelve-hour intervals as long as the temperature continued to rise.

Experimental data

TEMPERATURES OF GERMINATION

In the experiments with the sterilized grain, a slight rise in temperature occurred in all cases. The summarized results are presented in table I

TABLE I

TEMPERATURES PRODUCED IN 200-GRAM SAMPLES OF STERILIZED GERMINATING GRAIN

TIME	SAMPLES STERILIZED BY DRY HEAT AT 100° C. FOR 30 HOURS		SAMPLES STERILIZED BY IMMERSION IN ALCOHOLIC MERCURIC CHLORIDE SOLUTION FOR 15 MINUTES		
	OATS[1]	WHEAT[1]	OATS[1]	WHEAT[1]	BARLEY[2]
days	° C.	° C.	° C.	° C.	° C.
0	17.0	17.0	17	16.9	17.3
	18.0	16.8	17.8	16.8	17.5
1	18.8	17.0	18.2	17.6	17.8
	21.0	17.0	19.4	18.0	18.6
2	22.8	17.4	20.2	18.2	19.0
	23.8	17.6	20.8	19.6	19.6
3	23.6	18.2	21.4	20.0	20.4
	22.2	18.4	21.2	20.0	20.8
4	21.2	19.0	19.8	20.2	20.9
		19.6		20.2	20.7
		19.8		19.4	
		19.8		19.0	

[1] Composite of five samples.
[2] Composite of ten samples.

and figure 1. With the oats and wheat the data presented are a composite of five samples, while with the barley ten samples were used. The rise in temperature with sterile oats was from 17° C. to 23.8° C. on the third day after heat sterilization, and from 17° C. to 21.2° C. on the fourth day after chemical treatment. That is, the germination of oats raised the temperature of a 200-gram sample from 4.2° C. to 6.8° C. In the case of wheat, after heat sterilization the temperature rose from 17° C. to 19.8° C. in four days; after chemical sterilization from 16.9° C. to 20.2° C. in four days.
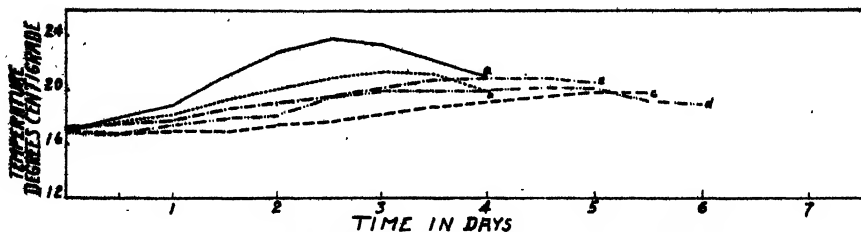
FIG. 1. Temperatures of germination of oats, wheat and barley after sterilization. a—oats sterilized by dry heat. b—oats sterilized by chemical means. c—wheat sterilized by dry heat. d—wheat sterilized by chemical means. e—barley sterilized by chemical means.

The germination of the wheat raised the temperature of a 200-gram sample from 2.8° C. to 3.3° C. With the barley the temperature of germination after chemical sterilization caused a rise of 3.6° C. for a 200-gram sample or a rise from 17.3° C. to 20.9° C. in four days. These data indicate that the high temperatures found in heating grain could not be caused by germination alone, since in no case did the temperature rise above 25° C.—a temperature which was found in a single case among the trials with oats.

Temperatures due to fungous growth

In order to select proper fungi for heat production in grain, a preliminary survey was made of the species of molds present on "heating" grain and three of those commonly found in such materials were selected. They were: *Aspergillus niger* Van Tieghem, *Aspergillus flavus* Brefeld, and *Aspergillus fumigatus* Fresenius. These three species had been isolated in pure culture and grown on corn-meal agar slants. From these slants, subcultures were made to corn-meal in Erlenmeyer flasks and from these cultures the spores were gathered for inoculating the grain in the thermal chambers. Thus all medium was eliminated from the chamber. Wheat, oats and barley were used in the trials. The seeds were sterilized by dry heat in 200-gram samples as in the case of those studied for temperatures of germination. After sterilization the seeds were allowed to cool to room-temperature and then a sample of the grain was removed from the container and its moisture content determined by the Brown-Duvel method. A germination test was made on a second portion and all samples testing less than 90 per cent. germination were discarded. Those samples of proper germinability were then brought to 18 per cent. moisture content by the addition of sterile water and placed in the ice box for 24 hours to allow the added water to diffuse throughout the mass. After this time, the samples were transferred to sterile Dewar flasks prepared in the same way as those used for determining the heat of germination. Spores from the cultures

TABLE II

TEMPERATURES PRODUCED IN 200-GRAM SAMPLES OF STERILIZED GRAIN WITH A MOISTURE CONTENT OF 18 AND 20 PER CENT. BY THREE SPECIES OF *Aspergillus*

	Oats				Wheat						Barley		
Time	*Aspergillus niger* AT 18% MOISTURE	*Aspergillus flavus* AT 18% MOISTURE	*Aspergillus fumigatus* AT 18% MOISTURE	CONTROL STERILE GRAIN	*Aspergillus niger* AT 18% MOISTURE	*Aspergillus niger* AT 20% MOISTURE	*Aspergillus flavus* AT 18% MOISTURE	*Aspergillus flavus* AT 20% MOISTURE	*Aspergillus fumigatus* AT 18% MOISTURE	CONTROL STERILE GRAIN	*Aspergillus niger* AT 18% MOISTURE	*Aspergillus flavus* AT 18% MOISTURE	CONTROL STERILE GRAIN
days	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
	25.9	25.1	26.8	26	25.7	28.1	28.1	29.7	26.0	25	28.1	33.4	29
	28.5	26.2	28.0	26	29.0	33.4	29.8	30.7	27.2	25	31.1	34.2	27
1	31.7	26.8	29.1	25	32.0	37.1	30.0	30.6	27.5	24	33.6	38.2	28
	36.9	29.2	30.0	25	34.2	41.4	30.4	30.4	28.2	22	38.4	37.4	27
2	42.4	35.5	31.5	24	35.0	45.6	32.5	30.8	28.2	22	41.9	37.7	28
	46.6	41.2	32.7	24	35.5	49.4	34.0	34.1	27.7	23	44.3	41.4	27
3	48.3	47.0	36.5	24	34.0	51.1	35.5	35.8	28.5	23	44.6	43.2	29
	51.2	50.6	40.4	24	31.7	51.2	35.2	38.5	29.2	22	43.6	47.0	27
4	52.2	51.1	45.9	25		51.5		—	29.0	21	44.8	46.2	27.5
5	50.8	50.8	49.5	25		51.2		44.8	30.5	21	46.6	47.7	26
	49.0	47.4	51.0	25		51.2		43.8	31.2		47.3	48.1	26
6	49.0		52.1	24		50.5		45.4			—	49.3	—
			52.6	24				45.5			48.4	49.4	24
7			53.2	25							49.2		27.5
											49.2		24
											50.0		27

FIG. 2. Temperatures induced in 200-gram samples of oats, wheat and barley by the growth of *Aspergillus niger, A. flavus* and *A. fumigatus* at 18 and 20 per cent. moisture content.

were introduced into the flasks at this time, being placed as near the center of the flask as possible by estimation. The introduction of the spores was made in a closed inoculation chamber. Temperatures were read and recorded as in the study of the heat of germination.

The data from the mold studies showed that these fungi were very active in producing heat when grown on moist grain. The summarized data are presented in table II and fig. 2. *Aspergillus niger* raised the temperature in the samples of the three grains at 18 per cent. moisture content used, as follows: oats, 25.9° C. to 52.2° C. or a total of 26.3 degrees; wheat, from 25.7° C. to 35.5° C. or a total of 9.8 degrees; and barley, from 28.1° C. to 50° C. or a total of 21.9 degrees. *Aspergillus flavus* raised the oats sample from 25.1° C. to 51.1° C. or a total of 26 degrees; wheat from 28.1° C. to 35.5° C. or 7.4 degrees; and barley from 33.4° C. to 49.4° C. or 16 degrees. *Aspergillus fumigatus* was used only with oats and wheat, and although it started somewhat slowly the rise in temperature was correspondingly large; oats, from 26.8° C. to 53.2° C. or 26.4 degrees; and wheat, from 26.0° C. to 31.2° C. or 5.2 degrees. In all cases the control flasks of sterile grain showed no appreciable rise in temperature during the experiments. In the case of wheat, two trials of five samples each were made using *Aspergillus niger* and *Aspergillus flavus* at 20 per cent. moisture content. The resulting increases in temperature with *A. niger* from 28.1° C. to 51.5° C. (23.4°) and *A. flavus* from 29.7° C. to 45.5° C. (15.8°) indicate that the amount of heat generated by the molds will vary with the percentage of water in the grain. Presumably there is a minimum, optimum and maximum which is determinable. Furthermore, the different molds differ in the time of their reaction. *A. niger* and *A. flavus* both act rather rapidly bringing the temperature to a maximum in approximately four days, while *A. fumigatus* did not reach its maximum heat production until after the fifth day.

That other fungi in addition to species of *Aspergillus* will produce heat in moist grain, is indicated from a trial made with a *Mucor* (sp. undet.) on oats at 18 per cent. moisture. In this trial four samples were run and the temperature was raised from 19° C. to 44° C. (25° C.) in six days.

One other significant fact may be deduced from these data; that is, the glumed grains, oats and barley, presented a better substrate for the growth of the fungi concerned than did the naked berry, wheat. This observation is borne out by the fact that in storage the glumed grains are considered to be more liable to heating than is wheat.

Summary

In order to study the effect of molds on the temperature of stored grain, three of those most frequently found in moldy grain, *Aspergillus niger* Van

Tieghem, *Aspergillus flavus* Bref., and *Aspergillus fumigatus* Fres., were grown in pure culture on artificial media and inoculated into samples of oats, barley, and wheat, which had been previously sterilized and brought to a moisture content of 18 per cent. The sterilized grain was placed in sterile, pint thermos bottles and the temperatures were obtained by means of thermometers inserted through sterile cotton plugs. Readings were made twice each day. The grain was sterilized by means of heat; 200-gram samples were held at 100° C. for 30 hours. Control experiments, consisting of samples of each grain used without the mold were placed at 18 per cent. moisture in separate flasks, and samples of sterilized grain with sufficient moisture to bring about germination, were carried out in all trials. In addition, samples of sterilized wheat at 20 per cent. moisture content inoculated with *A. niger* and *A. flavus* were included.

The data gathered showed that under the conditions of the trials, the rises in temperature due to germination of oats, wheat, and barley, were 6.8°, 2.6°, and 3.6° C., respectively. *A. flavus* raised the temperatures of oats, wheat, and barley at 18 per cent. moisture content, 26°, 7.4°, and 16° C., respectively. *A. niger* raised the temperatures of these grains 26.3°, 9.8°, and 21.9° C., respectively. *A. fumigatus* raised the temperature of oats at 18 per cent. moisture, 26.4° C. and wheat 5.2° C. At 20 per cent. moisture content, *A. flavus* and *A. niger* raised the temperature of wheat, 15.8°, and 23.4°, respectively. These facts strongly suggest the probability that in bins of stored grains marked increase in temperature may be ascribed to mold growth.

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LITERATURE CITED

1. ATANASOFF, D., and JOHNSON, A. G. Treatment of cereal seeds by dry heat. Jour. Agr. Res. **18**: 379–390. 1920.

2. BABCOCK, S. M. Metabolic water: its production and rôle in vital phenomena. Wisconsin Agr. Exp. Sta. Res. Bull. 22. 87–181. 1912.

3. BAILEY, C. H., and GURJAR, A. M. Respiration of stored wheat. Jour. Agr. Res. **12**: 685–713. 1918.

4. COHN, FERDINAND. Ueber thermogene Wirkung von Pilzen. Jahresber. Schles. Ges. vaterl. Cultur. **66**: 150–156. 1888.

5. ———————. Ueber thermogene Bacterien. Ber. deut. bot. Ges. **11**: (66)–(69). 1893.

6. DAANE, A. Moisture in combined wheat. Okla. Agr. Exp. Sta. Bull. 183. 1–15. 1928.

7. DARSIE, M. L., ELLIOTT, C., and PEIRCE, G. J. A study of the germinating power of seeds. Bot. Gaz. **58**: 101–136. 1914.

8. DUVEL, J. W. T. The vitality and germination of seeds. U. S. Dept. Agr. Bur. Pl. Indus. Bull. **58**: 1–96. 1904.

9. JAMES, L. H., RETTGER, L. F., and THOM, C. Microbial thermogenesis. II. Heat production in moist organic materials with special reference to the part played by microorganisms. Jour. Bact. **15**: 117–141. 1928.

10. KÖNIG, J., SPIECKERMANN, A., and BREMER, W. Beiträge zur Zersetzung der Futter- und Nahrungsmittel durch Kleinwesen. Zeitschr. Untersuch. Nahr. Genussmitt. **4**: 721–769. 1901.

11. ——————. Untersuchung landwirtschaftlich und landwirtschaftlich-gewerblich wichtiger Stoffe **1**: 607. 1923. (Fünfte Aufl.).

12. McHARGUE, J. S. The cause of deterioration and spoiling of corn and corn meal. Jour. Ind. Eng. Chem. **12**: 257–262. 1920.

13. MIEHE, HUGO. Ueber die Selbsterhitzung des Heues. Arb. deut. landw. Ges. **196**: 1–36. 1911.

14. MORGENTHALER, OTTO. Über die Mikroflora des normalen und muffigen Getreides. Landw. Jahrb. Schweiz. **32**: 551–572. 1918.

15. NABOKICH, A. J. Über den Einfluss der Sterilisation der Samen auf die Atmung. Ber. deut bot. Ges. **21**: 279–291. 1903.

16. PEIRCE, G. J. The liberation of heat in respiration. Bot. Gaz. **53**: 89–112. 1912.

17. QUAM, OLAF. Kornets Aanding. En Relation Mellem Spireevne og Aandingsintensitet. Tidsskr. Norske Landbr., Aarg. **13**: 263–284. 1906.

18. THOM, C., and CHURCH, M. B. The Aspergilli. 272 pp. Baltimore. 1926.

19. ——————, and LE FEVRE, EDWIN. Flora of corn meal. Jour. Agr. Res. **22**: 179–188. 1921.

CALLUSING OF COTTON STEM CUTTINGS

H. E. REA

(WITH PLATE)

In a previous paper (6) the ability of cotton stem cuttings to callus and root was reported. In this earlier experiment rather meager results were obtained. Only 43 per cent. of the cuttings used callused, while barely 6 per cent. rooted. A number of trials have subsequently been made without materially increasing the percentages of cuttings rooted.

The stem cuttings of some plants do not necessarily callus before forming roots but, in general, callusing is associated with the rooting of stem cuttings (4, 7). All of the stem cuttings of cotton that have been observed to root have first callused so that the formation of calluses may probably be considered an essential accompaniment to the rooting of cotton cuttings. Thus little hope is entertained for obtaining a very high percentage of rooting before a higher success at callusing cotton stem cuttings is secured. With much the same idea that germination tests are conducted with seed, it was decided to make a number of callusing trials with cotton stem cuttings. While the securing of callus does not insure the rooting of a cutting ordinarily, the factors that favor callusing also favor rooting (4, 7). From such callusing experiments it was hoped that a better idea of the optimum conditions for rooting cotton stem cuttings might be more rapidly determined.

Early in the fall of 1929 some Osage-orange (*Toxylon pomiferum*) stems enclosed within an ordinary glass fruit jar, which had been partially filled with moist soil before closing, was observed to have callused very profusely. This was not considered unusual for Osage-orange stems but it did suggest the use of this type of jar as an interesting propagating chamber. When glass jars are loosely closed with the screw top, they provide most of the features of the Wardian propagating case (3) in a unit very convenient for experimental work.

Accordingly, on November 13, 1929, a number of callusing trials, using the glass jar as a propagator, was set up. The process of preparing the material and setting up the experiment consumed the three days prior to the thirteenth, but all of the propagators were not finally in place until that day. From previous experience it was known that handling the cuttings during the process of callusing was injurious so that few observations were made prior to the completion of the test. Previous experience also indicated that approximately four weeks were required for the cuttings to callus so that it was decided to allow six weeks for the completion of the

experiment. On December 22 and 24 all propagators were opened and
records made of the number of cuttings with clean basal callus within each
propagator chamber.

A certain procedure was established as standard on the basis of usual
recommendations (1, 3, 9). These standard practices were used with the
bulk of the cuttings handled, while variations from these standards were
made from group to group. These standards and variations made are de-
scribed below.

Plant materials

CUTTING WOOD

In collecting the cotton plants from the field all the leaves and lateral
branches were trimmed from the central stalk. Only the main stem was
used in making cuttings. As a usual procedure the main stem was divided
roughly into three sections: the upper one-third furnishing the soft wood
cuttings; the middle one-third the medium mature cuttings; and the lower
one-third the mature cuttings. In the case of the soft wood cuttings this
division was not always strictly held to, as portions of the upper one-third
were sometimes too mature to be classed as soft wood. In no case was any
wood used that was so tender as to wilt down under the conditions of the
experiment.

All cutting wood was used immediately upon collecting it from the field
except in one lot where the storage of the wood for twelve hours was tested.
No special treatment was given the wood under storage as it was held at
dry air condition in the laboratory. Fresh cuts were made on the wood
stored as the cuttings were placed in the propagators.

STANDARD CUTTINGS.—The standard cutting used throughout the ma-
jority of the tests consisted of stems from 10 to 15 centimeters in length con-
taining from 3 to 4 nodes. Both the apical and the basal cuts were made
with a sharp knife and the basal cut was made closer to the node than the
apical cut. All standard cuttings were cleaned of any buds, bracts and
leaves which were not removed in the field. All cuts made with the knife
were sloping or angular. In specific experiments variations from the stand-
ard cuttings were made to test the particular variation.

TWO NODE CUTTING.—The short cuttings were from 5 to 7 centimeters
in length and included a single internode with 2 nodes. The cuts were
made as with the standard cutting.

ROUGH CUTS.—Cuttings made of both standard and 2 node size, where
the cuts were made with sharp pruning shears and only the leaves, buds,
and bracts removed in the field trimming, were classed as rough cuts. All
cuts made with the shears were straight or square.

EMBEDDING OF CUTTINGS

In using standard length cuttings placed in soil, the base of the cutting was usually covered to a depth of about 5 centimeters. In one series the amount of soil itself was varied. In these experiments the cuttings were covered from 4 to 8 centimeters in accordance with the volume of soil used. When *Sphagnum* moss was used the butts of the cuttings were covered less than 4 centimeters. In the case where no solid medium was added the cuttings rested on the bottom of the propagator.

In a number of propagators the bases of standard cuttings were inverted and left exposed in the moist air above the soil material used. The tops of the cuttings in these jars were embedded in the soil about 5 centimeters. Inverted cuttings were not used with media other than soil materials.

The two node cuttings were too short to permit covering the bases sufficiently to hold them erect so that they were allowed to lie flat on the surface of the soil material, leaving the entire cutting exposed. With other propagators in which two-node cuttings were used the cuttings were completely covered with the soil material.

POTASSIUM PERMANGANATE TREATMENT

Curtis (2) was able to hasten the rooting of *Ligustrum ovalifolium* by various treatments of potassium permanganate. To test the possible value of this chemical in stimulating callusing in cotton one hundred and forty cuttings were treated for thirty minutes. The basal cuts of four separate lots of thirty-five cuttings each were submerged in 2, 1, 0.5, and 0.25 per cent. solution of potassium permanganate in water for thirty minutes and then washed with water before placing them in the propagators. As a check against the potassium permanganate treated cuttings one lot of thirty-five cuttings was allowed to stand in tap water for the same length of time, under similar conditions. In each propagator in these tests five cuttings of untreated *Ligustrum japonicum* were also included as a check against the general condition for callusing within the propagator.

All the cuttings in these experiments were placed in ordinary quart fruit jars along with such other materials as are described under media. The jars were closed by screw tops without any rubbers. No attempt was made to modify the light condition under which propagators were placed. The fruit jar gives in a small unit propagation cases very similar to the Wardian propagating case when the top is loosely screwed on. The normal number of cuttings placed within each propagator was 10. To determine results of crowding the cuttings, twenty cuttings per propagation were used in a number of cases.

Media

SOIL MATERIAL

FINE GRAVEL.—The fine gravel material used contained rounded quartz pebbles ranging in size from slightly more than one fourth inch in diameter to slightly more than one fourteenth of an inch. The screening of the fine gravel through various sized screens showed that 22 per cent. by weight failed to pass through the screen with 4 meshes to the inch; 63 per cent. was held back by a screen with 6 meshes, and 15 per cent. by a 14-mesh screen. All of the material passed readily through a 2-mesh screen while none passed through the 14-mesh screen. The moisture content of this material at the beginning of the experiments was 2.7 per cent. and no water was added during the course of any of the trials.

COARSE SAND.—The coarse sand consisted of much finer quartz particles most of which were angular, although a few of the largest particles were rounded. A screen with 6 meshes retained 11 per cent. by weight of the larger particles; while 54 per cent. passed through a screen of 12 meshes but were retained by the 14-mesh screen. All of the material passing through the 14-mesh screen was retained by the 20-mesh screen, and made up 35 per cent. of the total weight of the composite samples of coarse sand. The coarse sand continued 4.6 per cent. moisture.

FINE SAND.—The fine sand contained particles which passed through the 12-mesh screen but failed to pass through the 20-mesh to the extent of 10 per cent. by weight. The material that passed the 20-mesh screen but failed to pass through the 40-mesh screen made up 85 per cent. of the samples while 5 per cent. passed through the 40-mesh screen but was retained by the 60-mesh screen. This material contained 8.2 per cent. moisture.

HOUSTON BLACK CLAY.—This material was surface soil of typical Houston Black Clay and contained 28.2 per cent. moisture. In this condition it was very friable.

VOLUME OF SOIL MATERIAL.—In sections of the experiment the volume of soil was varied. The jars were filled at the rates of 200, 350, and 500 milliliters of soil in the various sections of the test. The total value of the jars was found to be 965 milliliters so that 765, 615, and 465 milliliters of the space in the jars above the respective volumes of soil were occupied by air.

SOIL MOISTURE.—The moisture content of each material used was determined at the beginning of the experiment and is given under the description of the specific material. Unfortunately, determinations of the final moisture of the soil at the end of the experiment were not made. However, the phenological studies of the cuttings indicated that little or no moisture

was lost from the propagation chamber during the test period. In order to furnish some evidence to support these observations a separate set up was tested later. On February 20 moisture determinations were made on four separate lots of Houston black clay and one lot of coarse sand. These lots were divided into four separate fractions and treated as shown in table I for six weeks and the determination of moisture made at the end of that time.

TABLE I

AVERAGE PER CENT. OF MOISTURE IN HOUSTON BLACK CLAY AND COARSE SAND AFTER
STORAGE IN LOOSELY CLOSED GLASS JARS FROM FEBRUARY 29–APRIL 4, 1930

LOCATION FROM FEB. 20– APR. 4	HOUSTON BLACK CLAY		COARSE SAND
	JARS FILLED 3/4 FULL	JARS FILLED 1/4 FULL	JARS FILLED 3/4 FULL
INITIAL PER CENT.	37.7*	37.7	3.8
WEATHER PLAT 9″ UN- DERGROUND	38.3	....	......
ROOM NO. 1	37.6	37.6	3.7
GREENHOUSE	38.7	....	......

* The average percentage of moisture recorded in the table is the result of four separate determinations.

SPHAGNUM MOSS

The Sphagnum moss used was the common commercial grade, unsterilized. The moss was thoroughly saturated and then squeezed as dry as possible by hand.

In several series no medium other than the air contained within the jar was used. In one series both the cuttings and jar were washed with tap water and immediately drained. In this series such water as clung to the jar and cuttings was left in the jar. In a second series the unwashed cuttings were placed in jars that had been previously washed in tap water. In a third set up both the cuttings and jar were left without introducing any free water.

Temperature

Although the temperature could not be controlled directly for the benefit of this experiment, four locations were available where daily temperature records were being kept. In table II is given the weekly mean and also the maximum and minimum temperatures for each location throughout the course of the experiment.

TABLE II

AVERAGE AND EXTREME TEMPERATURES PREVAILING THROUGHOUT THE EXPERIMENT

DATE WEEK ENDING	AVERAGING TEMPERATURE			
	WEATHER PLAT 9″ UNDERGROUND	ROOM NO. 1	ROOM NO. 2	GREENHOUSE
	° C.	° C.	° C.	° C.
Nov. 16	16.0	16.0	14.0	16.0
Nov. 23	12.0	15.0	17.0	15.0
Nov. 30	9.0	14.0	16.0	21.0
Dec. 1	9.0	15.0	17.0	21.0
Dec. 14	9.0	21.0	21.0	27.0
Dec. 21	14.0	17.0	18.0	21.0
Average	11.5	16.3	17.3	20.2
Maximum ...	18.0	28.0	28.0	36.0
Minimum	4.0	3.0	3.0	4.0

Results

The percentage of cuttings callusing under the various conditions just outlined was generally low; but in a few cases the results were excellent. The data concerning the percentage of callusing in the various trials are given in table III.

Selection of the proper wood is considered very important in securing a high percentage of callusing with any kind of cuttings (5, 8). Some types of wood apparently will callus under a wide variety of conditions while other wood will callus only feebly under the most careful and specialized environment. Cotton stem cuttings appear to respond only to a narrow range of conditions. In previous trials soft wood of cotton has been callused in an environment under which mature wood has shown little tendency to callus. Yet under the environment provided in this recent experiment the reverse was true. Soft wood cuttings were callused in the propagators in only 6.1 per cent. of the trials while the propagation callusing was increased as more mature wood was used. Out of a large number of trials made 25.9 per cent. of the medium mature, and 46.8 per cent. of the mature cuttings produced calluses.

Immediate use of the cutting wood after it has been taken from the plant is usually recommended as conducive to the best results (1, 3, 9). The bulk of the cutting wood collected for these trials was used immediately after collecting but a small portion was stored for twelve hours prior to making the cuttings. The records in table III show that 31.7 per cent.

TABLE III

PERCENTAGE OF CALLUSING UNDER THE CONDITIONS TESTED

CONDITIONS OF EXPERIMENTS		TOTAL NO. CUTTINGS	CUTTINGS CALLUSED	
			NUMBER	PER CENT.
Cutting wood Condition Degree of maturity	soft	196	12	6.1
	medium	548	142	25.9
	mature	474	222	46.8
Freshness	12 hrs. storage	110	64	58.2
	immediate use	1204	382	31.7
Type of cut	rough	130	43	33.1
	smooth	1174	403	34.3
Position of cutting	upright, base covered	486	132	27.2
	inverted, base exposed	80	79	98.7
	horizontal, completey covered	40	21	52.5
	horizontal, completely exposed	263	160	60.8
Chemical treatment	water (check)	35	24	68.6
	2 per cent. $K_2MN_2O_8$	35	5	14.3
	1 per cent. $K_2MN_2O_8$	35	6	17.1
	0.5 per cent $K_2MN_2O_8$	35	11	31.4
	0.25 per cent $K_2MN_2O_8$	35	9	25.7
	Ligustrum (check)	175	168	96.0
Condition in propagating case	crowded, 20 cuttings each	400	194	48.5
	non-crowded, 10 cuttings each	490	185	37.8
Media for callusing	fine gravel	683	245	35.9
	coarse sand	115	52	45.2
	fine sand	146	23	15.9
	Houston black clay	115	66	57.4
Depth of soil	2 inches deep	823	289	35.1
	3 inches deep	161	70	43.5
	4 inches deep	40	5	12.5
Other media air in	Spaghnum moss	25	0	0
	jar and cuttings H_2O wash	60	5	8.3
	cuttings H_2O wash	40	5	12.5
	jar and cuttings dry	142	27	19.0
Temperature	buried underground 11.5° C.	180	0	0
	room No. 1 16.3° C.	430	79	18.4
	room No. 2 17.3° C.	380	112	29.5
	greenhouse 20.2° C.	220	82	37.3

and 58.2 per cent., respectively, of the immediately used, and the stored cuttings produced callus tissue. The actual percentage was in favor of storage. It is possible that the storage period improved the cutting wood. It is at least evident that delaying the use of the wood for twelve hours did not injure it.

A clean, smooth, sloping cut in preparing the material for propagation is favored in ordinary procedure (4). Such cuts were standard for most of the cutting wood handled; however, one lot of wood was crudely prepared with pruning shears. From the results obtained as presented in table III, it will be seen that about equal percentages of callusing were secured in the two groups. In the lots where cuts were carefully made with a sharp knife 34.3 per cent. of the cuttings callused, while in those prepared with less care with shears, 33.1 per cent. callused.

The placing of the cuttings in the medium is often manipulated to provide optimum conditions for callusing. In some instances hardwood cuttings are buried in moist sand beds with the base of the cuttings up (1, 3). This practice is especially common where sunlight is the only source of heat and the upper layer of the sand is warmer. Ordinarily, however, the cuttings are placed upright with the bases covered sufficiently with the medium to hold them erect and to prevent drying. Such procedure was the standard method of placing cuttings in this test. Several variations were also studied. In one case, the standard placement was completely reversed. The tops were buried in the medium and the base of the cuttings were left protruding in the air contained within the propagator. Several lots of short cuttings were used and as they could not conveniently be placed vertically they were allowed to take a horizontal position. One group of shorter cuttings was left exposed on the surface of the medium while another was completely covered. From a study of the section of table III, presenting the records of these variations in treatment, it is seen that 98.7 per cent. of the inverted long cuttings callused as compared with only 27.2 per cent. of those not inverted. Both groups of the shorter cuttings callused fairly well, as 60.8 per cent. of those completely exposed, and 52.5 per cent. of the cuttings completely covered callused. It should be noted that exposure to moist air in both cases gave the highest results. Wherever upright placement was followed, a great many of the exposed tops callused; however, such calluses are not considered in the present analysis. In the group of short cuttings callusing on both ends was general. There is little doubt that the moist air confined within the propagators above the soil material presented the optimum conditions for callusing.

Chemical treatment of the base of certain cuttings has stimulated callusing. By treating cuttings of *Ligustrum ovalifolium* with weak solutions of potassium permanganate CURTIS accelerated the development of roots (2). An attempt was made to test the possible value of potassium permanganate treatments with cotton cuttings. The bases of separate lots of cuttings were submerged with 2, 1, 0.5, and 0.25 per cent. solutions of potassium permanganate in water for thirty minutes before being placed in the propa-

gators. A similar lot of cuttings was treated with tap water for the same length of time. Calluses were developed on 68.6 per cent. of the water treated cuttings while 31.4 per cent. of calluses was the highest for any of the chemically treated group. To measure the general favorableness for callusing within the propagators used for the chemically treated cuttings, a number of untreated *Ligustrum japonicum* cuttings was introduced. That conditions were favorable for *Ligustrum* is indicated by the fact that 96.0 per cent. of these cuttings callused.

One factor concerning the propagators themselves was investigated. The standard number of cuttings used was ten per propagator but in one group 20 cuttings each were used. The percentages of cuttings callusing were 37.8 and 48.5 respectively for the 10 and 20 cuttings per propagator lots. Thus there appears to be no reason for not filling each propagator to capacity.

Of equal importance to any other factor is the medium in which cuttings are callused (1, 3, 9). For this purpose soil materials are extensively used, particularly, clean, sharp sand. The soil materials tested in this experiment included fine gravel, coarse sand, fine sand and Houston black clay. From table III it is evident that Houston black clay gave the best results. Cuttings placed in Houston black clay callused in 57.4 per cent. of the cuttings, while coarse sand, fine gravel, and fine sand induced callusing of 45.2, 35.9 and 15.8 per cent. respectively.

The volume of the soil in the propagator affects the result. When the propagators were filled with 200 milliliters of soil, 43.5 per cent. of the cuttings callused. With 350 milliliters of soil 35.1 per cent. callused. The use of 500 milliliters of soil seemed to interfere with callusing as only 12.5 per cent. of the cuttings used with this volume of medium callused. The volume of medium used was probably of little significance in itself but it is very likely that it influenced two other more important factors. When too little medium was used, the supply of moisture was probably lowered. With the use of too much medium the air space (available oxygen) was restricted. These two factors are very vital to the callusing of the cutting.

Although Sphagnum moss is usually highly recommended for callusing cuttings, we obtained very poor results with it. Not a single cutting callused where Sphagnum was used. Before the experiment had progressed very far, the poor results with Sphagnum moss were noted, and additional trials were made. Although various arrangements of cuttings in Sphagnum were tried, few cuttings were successfully callused.

Three separate lots of cotton cuttings were placed in propagators with no medium other than air. In one lot the cuttings and the interior of the propagator were washed with tap water. In another only the propagator

was washed, and in the third lot no free water was introduced. The results presented in table III clearly show that these were all less successful than the tests in which soil materials were used. Of the three series of propagators in which no solid medium was used the one in which no water was used gave the best results. In this lot 19.0 per cent. of the cuttings callused.

Temperature is one of the most vital factors affecting the callusing of cuttings (7, 1, 3, 9). One lot of cuttings was held at an average temperature of 11.5° C.; none of these callused. Three other lots were held at an average temperature of 16.3, 17.3 and 20.2° C., respectively. The percentage of cuttings callusing increased with increasing temperature, as is shown in table III.

Summary

Reviewing the results of the experiment on callusing cotton stem cuttings in glass fruit jar propagators, it is found that certain conditions provided were injurious. The use of soft wood cuttings was notably unsuccessful. The treatment of cotton cuttings with aqueous solution of potassium permanganate reduced the percentage of callusing in the concentrations tried. Spaghnum moss was found to be a very poor medium for cotton cuttings and only very few cuttings were callused without the use of soil media. An average temperature of 11.5° C. was too low to permit callusing.

Other conditions provided seemed to be optional. Cotton cuttings callus just as readily, and perhaps more so, after being stored for twelve hours as when used immediately. From the results obtained, it seems that smooth cuts are not entirely essential and that crowding of cuttings in the propagator is not injurious.

On the other hand several factors seem to be highly favorable in callusing. Mature wood gave results superior to medium mature and soft wood. The exposure of the base of the inverted cutting to the moist air above soil materials appears to provide good conditions for callusing. Houston black clay was more favorable to callusing than other soil material used whether the bases of the cuttings were covered or exposed. The highest temperature used (20.2° C.) produced the highest percentage of callusing.

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LITERATURE CITED

1. BAILEY, L. H. The nursery book. Macmillan Co. New York. 1912.
2. CURTIS, OTIS F. Stimulation of root growth in cuttings by treatment with chemical compounds. Cornell University Agricultural Exp. Sta. Memoir 14. 1918.

3. HOTTES, ALFRED C. Practical plant propagation. A. T. De La Mare Co. New York. 1927.

4. PRIESTLEY, J. H., and SWINGLE, CHARLES F. Vegetative propagation from the standpoint of plant anatomy. U. S. Dept. Agr. Tech. Bull. 151. 1929.

5. PROEBSTING, E. L. The relation of stored food to cambial activity in the apple. Hilgardia 1: 81–106. 1925.

6. REA, H. E. Asexual reproduction of cotton. Journal of Heredity 19: 357. 1928.

7. SWINGLE, CHARLES F. A physiological study of rooting and callusing in apple and willow. Jour. Agr. Res. 39: 81–128. 1929.

8. TRAUB, HAMILTON P. Regional and seasonal distribution of moisture, carbohydrates, nitrogen and ash in 2–3-year portions of apple twigs. Minnesota Agr. Exp. Sta. Tech. Bull. 53. 1927.

9. YERKES, GUY E. Propagation of trees and shrubs. U. S. Dept. Agr. Farmer's Bull. 1567. 1929.

EXPLANATION OF PLATE

A. Cutting wood used: 1. Soft. 2. Medium mature. 3. Mature. 4. The main stem of a cotton plant as trimmed in the field. 5. A defoliated cotton plant showing lateral branching.

B. Types of cuttings as to maturity of wood: 1. Standard length mature. 2. Medium, and 3, Soft wood cuttings.

C. Other types of cutting used: 1. Rough trimmed. 2. Smooth cuttings of standard length. 3. Short two-node cuttings.

D. Three cuttings with typical callusing of cambium along basal cut.

E. Two jars showing placement of standard cuttings in the propagating cases.

F. The calluses of cuttings inverted in the jar with basal cuts exposed.

G. A thoroughly sorted cotton cutting.
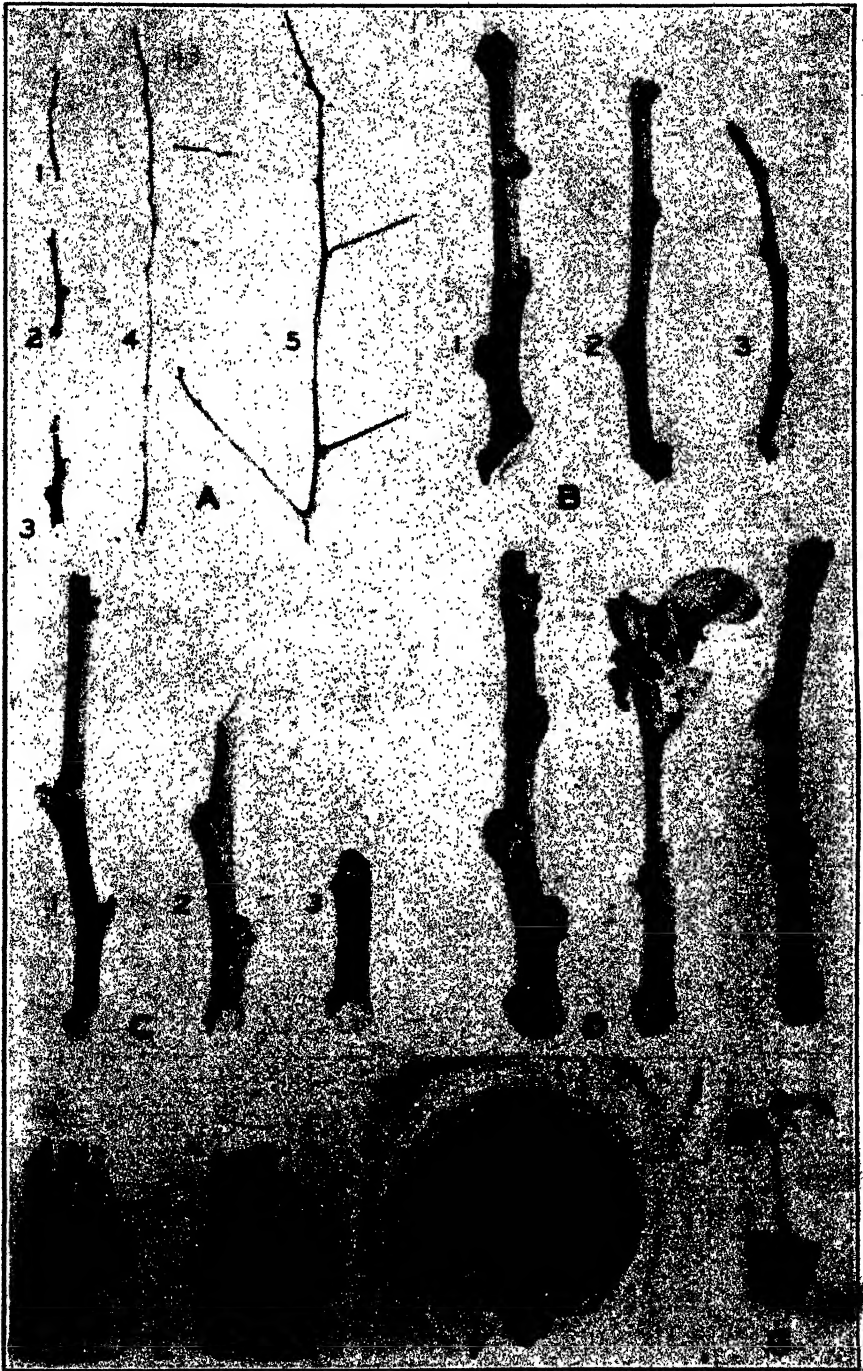
REA—CALLUSING OF COTTON

PRELIMINARY STUDY OF ELONGATION OF ROOTS OF GEORGIA COLLARDS AS AFFECTED BY SODIUM LUMINAL[1]

T. W. PRATT

(WITH THREE FIGURES)

No study has hitherto been reported in botanical literature as to the toxic effects of sodium luminal on plant growth. Apparently, all of the work with sodium luminal of biological character which has been reported, with the exception of medical cases, has been based upon animal experimentation. Interest in phytopharmacology and phytotoxicology has been stimulated by the experiments of MACHT and KRANTZ (7) who determined the effect of digitalis solutions on the growth of seedlings. They found that the inhibition of the root-growth of *Lupinus albus* was proportional to the concentration of the digitalis solutions employed.

Obviously, much information may be obtained by observing the behavior of plant tissue under the influence of sodium luminal. Since, however, no work on plant behavior in connection with sodium luminal, or luminal has previously been reported, it was considered advisable to simplify any problem selected and to eliminate all complicating factors possible, if practical. Georgia collards, a variety of *Brassica oleracea*, were chosen for the tests. They were the same kind of plants used by FARR (2) in his studies of the effect of solutions on the elongation of root hairs. The seed of Georgia collards was purchased from W. Atlee Burpee Co., Philadelphia, which company attested the purity of the seed.

The main objects of the work here reported are: (1) the observation of any physiological reactions on the elongation of the roots of Georgia collards as affected by sodium luminal; and (2) the determination of any correlation between the root elongation of the seedlings used in the experiments and the concentration of sodium luminal.

Procedure

The apparatus for the experiment was modified from that used by Farr (2) and set up as shown in fig. 1. Six microscopes, fitted with eyepiece micrometers calibrated in microns, were fixed rigidly on a platform placed on the bases of two iron supports. The barrels of the microscopes were arranged in a horizontal plane in order to allow the attachment of a glass water chamber to each stage.

FIG. 1. Apparatus for the study of elongation of roots of Georgia collards as affected by sodium luminal.

De Khotinsky's hard cement was employed to cement together the margins of six water chambers whose inside dimensions were, approximately, $5.5 \times 5 \times 0.6$ cm. An inlet and outlet for solutions, consisting of glass tubing, were placed in the top of each chamber. A water chamber was held in any desired position on the microscope stage by a burette clamp and a 0.5 inch rubber band.

Six seedling holders, one to fit into each of the water chambers, were constructed. Each approximated 0.5 cm. in thickness and was made from

3×1 inch micro-slides. A small groove, about 3 mm. in width, was provided for in the holders to help keep the seedling in vertical position for measurements.

An aspirator and KOH tower were improvised to control the oxygen and carbon dioxide content of the solutions. The aspirator was made in the following manner. Two pieces of rubber tubing, each about four feet in length and having an inside diameter of 0.75 inch, were attached to two connections of a glass T-joint. This T-joint was mounted near the top of a support by a burette clamp in such a way that the third connection to the aspirator bottles and KOH tower was in an elevated position. One of the four-foot lengths of rubber tubing was joined with a water line; the other rubber tubing of similar length led into a drain trough.

A Squibb separatory funnel, 500-ml., served as a KOH tower. It was mounted near the center of the apparatus to facilitate the distribution of the CO_2-free air to all of the solution bottles. To the separatory funnel was attached a thistle tube for the removal of waste.

One system of connections, consisting of rubber tubing, glass T-joints, glass Y-joints, metal stopcocks, and bent glass tubing of suitable lengths, led from the aspirator to the eight 1500-ml. aspirator bottles and to the KOH tower. During operation of the aspirator, the CO_2-free air was drawn from the separatory funnel, containing potassium hydroxide, to the bottles containing solutions, the rate being controlled by the adjustment of stopcocks and the rate of water flow through the aspirator.

Six aspirator bottles, one for each microscope, were connected to the water chambers by rubber tubing. Hoffman (screw compressor type) clamps were used to control the gravity flow of the solutions from the aspirator bottles to the water chambers. Glass tubing and a small funnel, placed beneath the outlet of the water chamber, carried the used solution to a drain trough. Two additional aspirator bottles, connected with the aspirator and KOH tower, were used for the aeration of distilled water oftentimes required for solutions.

In general, the experimental routine was as follows. About 50 seeds of Georgia collards were placed on a wet filter paper in a moist chamber and permitted to germinate, in preparation for a day's tests. Two days afterwards, distilled water was added to the aspirator bottles and aerated with CO_2-free air one or more hours before the seedlings were mounted. Six seedlings, having roots which were straight and near 10 mm. in length, were selected and attached individually to the seedling holders with beeswax. Each water chamber was washed out and filled from the aspirator bottle by the solution in which a seedling was placed. The seeds of the young plants were allowed to remain above the water level. The root tip of each seedling was then focussed under a low power lens and the water

chamber made fast to the stage of the microscope with a burette clamp. Frequently the water chambers were changed in position to hold the root tips in the fields of vision of the microscopes. If no growth occurred in one or two hours, the mount was replaced or discarded. Only a few seedlings were necessarily remounted.

Usually careful observation of the seedlings was made between 8:00 and 11:00 P. M. One to three controls were selected for each day's tests. Readings of the elongation of the roots of all the seedlings were made every 15 minutes for 3 hours. Occasionally a reading was not recorded if some interference was evident. During the first hour of the 15-minute readings, 200 cc. of each concentration of sodium luminal required, was prepared. Five minutes before the expiration of the first hour of regular observation, the flow of distilled water was stopped. The remainder of the distilled water, in those aspirator bottles which were to be used for the drug, was replaced by a sodium luminal solution. At the end of the hour after readings were recorded, the water chambers were washed out by a rapid flow of solution from the six aspirator bottles, after which the flow was adjusted to drop regularly.

Readings of root elongation of the controls, as well as of the seedlings subjected to sodium luminal, were taken for 1.5 hours. Five minutes before the completion of the above 1.5 hour period of time, the delivery tubes from the aspirator bottles were again clamped. The aspirator bottles containing the sodium luminal solutions were washed and supplied with aerated distilled water. After the customary procedure, all of the water chambers were flushed with distilled water. Readings were further recorded for 2 15-minute periods. On completion of a day's observation, the readings which were at first designated in terms of the divisions of the eyepiece scale were then converted into microns and recorded. The average elongation of the roots of all the seedlings was tabulated as shown in table I.

The general routine was slightly modified as the plan of the procedure required. The work was divided into 3 series, each part consisting of the tests performed with certain concentrations of sodium luminal. Each solution of the drug was expressed in per cent. as was done by MACHT and KRANTZ (7) and arranged thus: Series 1—0.2, 0.4 and 0.8 per cent; Series 2—0.1, 0.5 and 1.0 per cent. and Series 3—0.3, 0.6, 0.7 and 0.9 per cent. In connection with each series, controls were used for comparison, two or three water chambers being reserved for that purpose.

The rate of the elongation of the roots of 10 seedlings was observed in each concentration of sodium luminal. This study, including the arrangement of the apparatus, was started in December, 1929, and finished in May,

TABLE I

AVERAGE ELONGATION OF ROOTS OF GEORGIA COLLARDS DURING 15-MINUTE INTERVALS FOR A PERIOD OF 3 HOURS IN DISTILLED WATER AND SODIUM LUMINAL

DISTILLED WATER				CONCENTRATION	ELONGATION OF CONTROL IN DISTILLED WATER							
					SODIUM LUMINAL						DISTILLED WATER	
μ 133.1	μ 138.3	μ 139.5	μ 137.7		μ 138.0	μ 143.8	μ 144.2	μ 144.2	μ 146.6	μ 137.6	μ 137.3	μ 134.2
179.4	172.9	185.7	167.9	0.2 per cent.	101.5	77.6	64.1	60.2	38.0	35.4	154.5	96.1
169.3	162.6	166.7	171.3	0.4 per cent.	75.1	59.9	41.9	25.4	14.6	5.1	53.6	25.6
169.4	174.5	185.9	193.6	0.8 per cent.	53.4	29.2	6.3	3.2	-9.0	-25.4	-2.4	12.9
178.7	187.0	190.2	213.1	0.1 per cent.	123.0	106.5	107.8	110.3	88.6	87.2	170.4	133.7
180.3	185.4	190.2	209.5	0.5 per cent.	72.7	49.8	40.8	39.5	15.2	26.5	137.6	81.7
177.0	181.1	184.7	196.2	1.0 per cent.	29.3	21.6	10.2	7.6	-0.6	-5.7	67.1	16.9
143.8	161.0	158.5	196.4	0.3 per cent	71.5	43.9	63.5	35.3	40.7	53.9	169.6	181.3
154.1	159.3	165.6	192.5	0.6 per cent.	48.9	49.7	42.0	25.4	19.0	25.5	131.8	65.1
121.9	180.2	138.1	190.0	0.7 per cent.	42.2	28.1	18.9	28.1	13.9	11.8	124.3	63.3
192.4	138.1	141.1	151.1	0.9 per cent.	17.8	22.8	12.6	3.1	4.4	4.8	93.3	29.5

TABLE II

AVERAGE RATE OF ELONGATION OF ROOTS OF GEORGIA COLLARDS GROWN AT FIRST IN DISTILLED WATER, THEN IN SODIUM LUMINAL, AND AGAIN IN DISTILLED WATER

EXPERIMENTS	ELONGATION IN DISTILLED WATER					ELONGATION IN SODIUM LUMINAL SOLUTIONS				ELONGATION ON APPLICATION OF DISTILLED WATER FOLLOWING SODIUM LUMINAL [3]	
	DURATION OF APPLICATIONS				COMPARATIVE ELONGATION [1]	CONCENTRATION USED	DURATION OF APPLICATION		COMPARATIVE ELONGATION [2]	FIRST 15 MINUTE PERIOD	SECOND 15 MINUTE PERIOD
	1 HOUR	AVERAGE, 15 MINUTES	1.5 HOURS	AVERAGE, 15 MINUTES			1.5 HOURS	AVERAGE 15 MINUTES			
	μ	μ	μ	μ	per cent.	per cent.	μ	μ	per cent.	per cent.	per cent.
Control	548.72	137.18	854.67	142.44	103.83						
1	769.22	192.30				0.1	799.5	133.25	69.29	88.56	69.55
2	706.0	176.5				0.2	378.8	63.13	35.58	87.50	54.49
3	659.83	164.95				0.3	309.02	51.5	31.27	102.81	79.59
4	670.0	167.5				0.4	223.8	37.3	22.27	32.03	15.27
5	765.6	191.4				0.5	286.52	47.75	24.94	71.89	46.68
6	671.5	167.87				0.6	210.76	35.1	20.9	78.51	38.82
7	630.25	157.56				0.7	143.27	23.87	15.14	78.89	40.21
8	723.2	180.8				0.8	58.7	9.78	5.409	-1.36	7.17
9	562.86	140.7				0.9	65.58	10.93	7.75	66.33	21.01
10	759.24	189.81				1.0	56.24	9.37	4.92	35.87	8.93

[1] The average rate for 15 minutes in column 3 is considered the basis of comparison = 100 per cent. elongation. Columns 4, 5 and 6 show with distilled water, how the sodium luminal effects are calculated.

[2] The per cent. of elongation is determined as the ratio of the average elongation in sodium luminal for 15 minutes, to the elongation in water for 15 minutes, in column 3. Thus $133.25 \times 100 \div 192.3 = 69.29$ per cent.

[3] These percentages are also based upon the averages of column 3, which are made the standard of comparison (= 100 per cent.)

1930. The readings, however, were taken over a period from March 3, 1930 to April 21, 1930.

Results

In table I, the control is based on the readings of all the seedlings in the different series not affected by sodium luminal. The percentages represent the different concentrations of sodium luminal employed, and their arrangement in the table is according to the order followed in the different series. The average elongation of the roots in microns, as recorded in the first four columns of the treated seedlings, was made in distilled water; the figures listed in columns 6, 7, 8, 9, 10, 11, dealing with the same plants, represent the growth as influenced by different strengths of the drug, and the averages in the last two columns of table I stand for the elongation observed during the second application of distilled water.
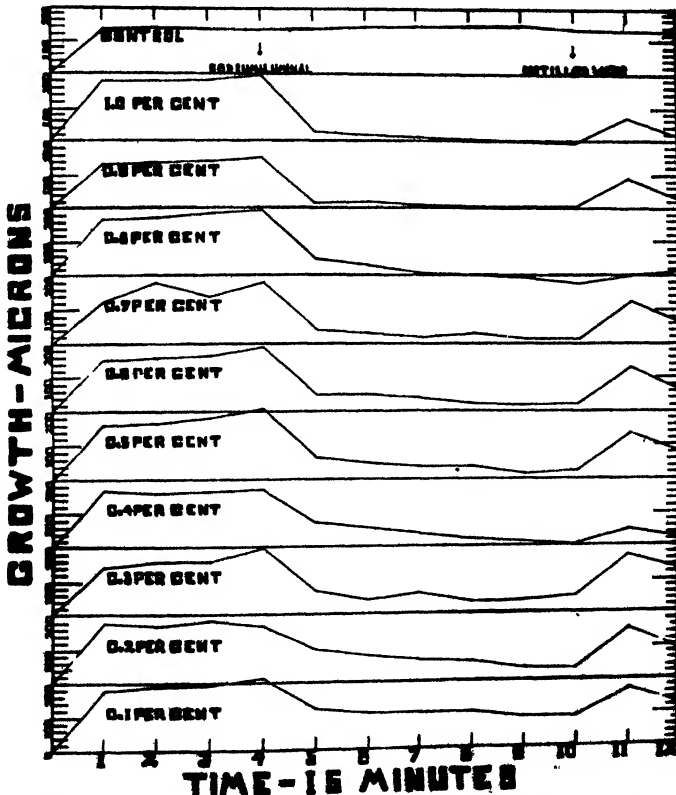
FIG. 2. Rate of elongation of the roots of Georgia collards in microns for 15-minute intervals during a period of 3 hours. Curves plotted from the averages of root-elongation taken from table I. Divisions on the ordinates represent elongation in microns; abscissas, corresponding with the bases of the graphs, are divided into equal parts which represent 15-minutes intervals of time.
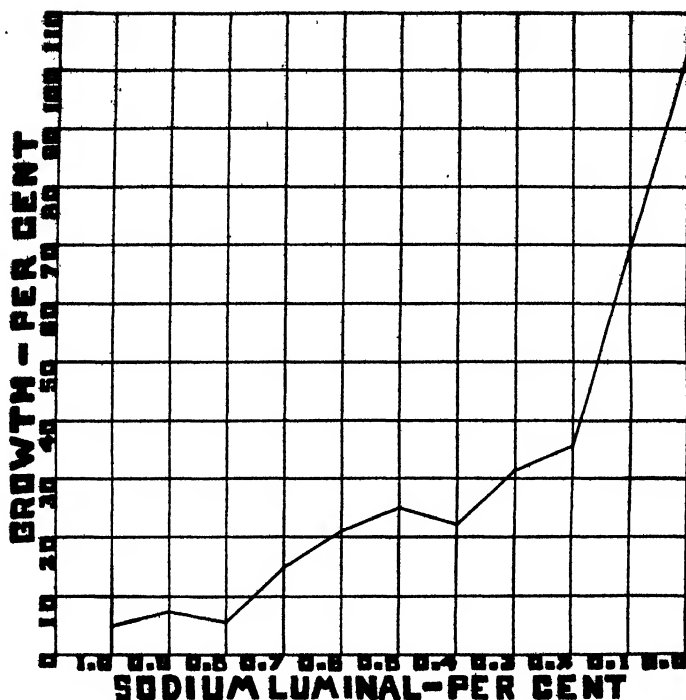
FIG. 3. Curve showing the per cent. of the elongation of the roots of Georgia collards grown in sodium luminal as compared with growth in distilled water. From data of column 10 and column 6 in table II. The percentages of elongation are represented by points on the ordinates. Spaces on the abscissas indicate the per cent. concentrations of the sodium luminal.

Discussion

Distilled water was found to be practical for use as a culture solution in these experiments. A few tests demonstrated that sodium luminal could not be used in Knop's nutrient solution as a precipitate would form when the two were mixed. Since the roots of Georgia collards made reasonably constant elongation in distilled water for a few hours, a nutrient solution was not considered necessary.

It is well known that distilled water is not suitable, in general, for plant cultures. TRUE (10) reported that the radicles of *Lupinus albus* were very sensitive to distilled water. TRUE and BARTLETT (12) found that the roots of *Lupinus albus* gave up their salts until the reserves were exhausted, when grown in the dark in distilled water. In referring to the effect of distilled water on the roots of certain seedlings, OSTERHOUT (8) states that "The effects which have been described as due to distilled water were also produced by water taken directly from ponds, rivers, springs; they are not due, therefore to toxic substances resulting from the

process of distillation." However, TRUE (10), in alluding to a paper published by TRUE and BARTLETT (11), claimed that Canada field peas make a fairly healthy growth in distilled water, which indicates that not all plants are sensitive in the same degree to distilled water.

The results of experiments conducted by HIBBARD (3) indicate that the seedling roots of *Lupinus albus* excrete a substance that inhibits growth. To prevent the possibility of such excretions modifying the behavior of the roots of Georgia collards, a flowing solution, which would remove toxic substances, was employed.

Due to the fact that a nutrient solution could not be used satisfactorily in connection with sodium luminal, root hairs were eliminated as possible test objects. Only relatively few plants, according to FARR (2) produce root hairs in solutions. Although Georgia collards will produce aquatic root hairs in a well-balanced nutrient solution, no similar development occurs in distilled water. Actually, there is a tendency for the amphibious root hairs of Georgia collards to become abortive when immersed in distilled water.

The use of root hairs would no doubt offer some advantages in determining the effects of certain drugs on plant growth. Root hairs are very sensitive and development is relatively simple, as explained by FARR (1). JEFFS (4) who investigated the relation of the elongation of root hairs to light and temperature, recognized that the response of root hairs could be more accurately measured than the complex results occurring in a multicellular structure such as a root.

Acceptable work, however, has been accomplished by the action of various chemical agents on the retardation of root growth. TREALEASE and TREALEASE (9) claim that root elongation in very young seedlings is not too complex for reliable, experimental results. In addition, MACHT and KRANTZ (7) obtained most consistent results in demonstrating that the root growth of the seedlings of *Lupinus albus* was inversely proportional to the concentration of the solutions of digitalis used.

No provision was made for the control of temperature. Variations in temperature may cause retardation or acceleration of root elongation. LEITCH (6) reported that the rate of growth of the roots of *Pisum sativum* can be expressed as a uniform curve from $-2°$ to about $29°$. Work performed by LEHENBAUER (5) as abstracted by TREALEASE (9) shows that VAN'T HOFF'S rule, that there is a doubling or trebling of chemical action by each rise in temperature of $10°$ C., applies to the growth of maize seedlings, within the range of 20 to $30°$ C.

The widest range of temperature (recorded about every hour) during the course of these experiments, was from 19.5 to $28°$ C. Such extremes of temperature were observed only twice. Usually the thermometer varied

from 22 to 24° C. Although controlled conditions would have been preferable, the fluctuations affect the seedlings equally in any given series of tests.

The disadvantages of working under variable light conditions were partially offset by employing three or more concentrations in each series. Any variations of root elongation of the plants within a series, based on a number of tests, are evidently due to the differences in the concentration of the sodium luminal.

No attempt is made to compare the effects produced by sodium luminal on root-elongation as shown in the different series, since influential factors such as temperature, light, humidity, etc., were not controlled.

The writer wishes to express his appreciation to Dr. R. E. Jeffs, plant physiologist, who ably directed the problem, and to acknowledge his indebtedness to Dr. Paul B. Sears, Head of the Department of Botany, who generously supplied the necessary materials, and offered many constructive criticisms.

Summary

1. Distilled water was found suitable as a plant culture for a preliminary study of the root-elongation of Georgia collards as affected by sodium luminal.

2. Sodium luminal, 0.1 to 1.0 per cent. was toxic to the roots of Georgia collards.

3. Pronounced retardation of root-elongation was produced by sodium luminal, used in concentrations from 0.1 to 1.0 per cent., within 1.5 hours after administration.

4. The effect of the sodium luminal on the root-elongation was immediate, the greatest retardation occurring during the first of the six 15-minute intervals of observation.

5. Distilled water partially removed the depressant action of the sodium luminal and accelerated the elongation of the roots.

6. In each series of experiments conducted, a close relation appeared between the elongation of the roots and the concentration of the sodium luminal. The stronger the concentration of the drug, the greater the retardation of root growth.

LITERATURE CITED

1. FARR, CLIFFORD H. The formation of root-hairs in water. Proc. Iowa Acad. Sci. 32: 157–165. 1925.

2. ——————. Studies on the growth of root hairs in solution. I. The problem, previous work, and procedure. Am. Jour. Bot. 14: 446–456. 1927.

3. HIBBARD, R. P. The question of the toxicity of distilled water. Amer. Jour. Bot. 2: 389–401. 1915.

4. JEFFS, R. E. The elongation of root hairs as affected by light and temperature. Amer. Jour. Bot. **12**: 577–606. 1925.

5. LEHENBAUER, P. A. Growth of maize seedlings in relation to temperature. Physiol. Res. **1**: 247–288. 1914. Abstract in Bot. Centralbl. **129**: 662–663. 1915.

6. LEITCH, I. Some experiments on the influence of temperature on the rate of growth in *Pisum sativum*. Ann. Bot. **30**: 25–46. 1916.

7. MACHT, DAVID I., and KRANTZ, JOHN C. Phytopharmacology: The effect of *Digitalis* solutions on the growth of seedlings. Jour. Pharm. and Exp. Therap. **31**: 11–25. 1927.

8. OSTERHOUT, W. J. V. Protoplasmic contractions resembling plasmolysis which are caused by pure distilled water. Bot. Gaz. **55**: 446–451. 1913.

9. TRELEASE, SAM F. and TRELEASE, HELEN M. Growth of wheat roots in salt solutions containing essential ions. Bot. Gaz. **80**: 74–83. 1925.

10. TRUE, RODNEY. The harmful action of distilled water. Amer. Jour. Bot. **1**: 255–273. 1914.

11. ————, and BARTLETT, HARLEY HARRIS. Absorption and excretion of salts by roots as influenced by concentrations and composition of culture solutions. I. Concentration relations of dilute solutions of calcium and magnesium nitrates to pea roots. Bur. Plant Industry. U. S. Dept. Agr. Bull. 231. 1912.

12. ————, and BARTLETT, HARLEY HARRIS. The exchange of ions between the roots of *Lupinus albus* and culture solutions containing one nutrient salt. Amer. Jour. Bot. **2**: 255–278. 1915.

EFFECT OF VARIOUS METHODS OF STORAGE ON THE CHLOROPHYLL CONTENT OF LEAVES[1]

Philip A. Harriman

(WITH ONE FIGURE)

Problems involving the quantitative determination of the chlorophyll content of leaves have been carried out chiefly with plants grown in a greenhouse or in the immediate vicinity of a laboratory. This allows the extraction of the chloroplast pigments within a few minutes after the samples are secured and, as the chlorophyll content of the leaves is practically unaltered, the results may be taken to represent the conditions existing in the living tissue. A recent investigation by Deuber (1) shows a wide variation in the chlorophyll content of the leaves of different species of trees. The chlorophyll determinations were all made from fresh leaves gathered from trees growing near the laboratory. It would be desirable to have similar information regarding the leaves of trees growing in other localities where environmental conditions are different. The investigation of Sprague and Shive (9) on the relations between chloroplast pigments and the dry weights of tops in dent corn and the work of Guthrie (3) on the effect of environmental conditions on the chloroplast pigments suggest interesting and instructive problems that might be carried out with plants growing in widely separated habitats.

In such cases the extraction and determination of the pigments must be made from stored or preserved leaves. As the question of storage of green tissues for later determination of chloroplast pigments has received only slight attention, it is the purpose of the present investigation to determine the effect of various methods of storage on the chlorophyll content of leaves.

Procedure

About 50 experiments were carried out with duplicate and in some cases triplicate samples. Since only a limited number of determinations could be made at the same time, it was necessary to divide the experiments into a number of series. In each series one set of samples was used for immediate extraction to determine the chlorophyll content of the fresh leaves; another set of samples was used to determine the moisture content of the leaves; and the rest were dried or stored under the various conditions of the respective experiments. The chlorophyll content of the dried or stored leaves was then determined and the amount retained expressed in per cent. of the chlorophyll content of the fresh leaves. The determinations were all made

on Wilson soy bean or on dwarf nasturtium leaves and, in most cases, on both.

The chloroplast pigments were extracted and separated after the method of WILLSTÄTTER (10) as modified by SCHERTZ (7, 8) and the amount of chlorophyll determined colorimetrically by means of a Duboscq colorimeter. The probable error of a single observation by this method is stated to be about 3.3 per cent. for chlorophyll solutions with concentrations of 0.05 grams per liter. In the present investigation the average probable error determined by Bessel's formula, P.E.M. equals $.6745\sqrt{\dfrac{\Sigma d^2}{n(n-1)}}$, is 2.16 per cent. Both pure chlorophyll[2] solutions and the artificial color standard of GUTHRIE (2) were used, but because of its greater stability it was found advantageous to use the artificial standard for most of the determinations.

To determine the effect of the temperature of storage on the chlorophyll content of the leaves, they were dried in ordinary drying ovens at 98° C., 72° C., 60° C., 45° C., 30° C., and at room temperatures; they were stored in an electric refrigerator at 5° C. and at −5° C.; and they were frozen with Dry Ice (solid carbon dioxide snow). In every case the leaves were spread on coarse filter paper in a single layer.

In order to bring about a rapid removal of the water from the leaves without increasing the temperature, a vacuum desiccator was used. The desiccator was evacuated by means of a filter pump and both anhydrous calcium chloride and concentrated sulphuric acid were used as dehydrating agents. A number of leaf samples were also desiccated at reduced pressure in the absence of oxygen and carbon dioxide, the desiccator being filled with air which had passed through a solution of potassium hydroxide and pyrogallic acid.

Discussion of results

EFFECT OF TEMPERATURE

When dried at 98° C. soy bean leaves retained only 30 per cent. of the chlorophyll found in the fresh leaves, but when dried at 72° C. they retained 88 per cent. of their chlorophyll. With further decreases of the temperature of drying, still higher percentages of the chlorophyll were retained, and at 45° C. 98 per cent. or practically all of the chlorophyll remained in the dried leaves. Soy bean leaves dried at 30° C., however, suffer a slight loss of chlorophyll and those dried at room temperature (approximately 20° C.) retain only 70 per cent. of their chlorophyll. It is of interest to note that leaves dried at this temperature retain less chlorophyll than those

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[2] The pure chlorophyll used was obtained through Dr. CARL G. DEUBER from Dr. F. M. SCHERTZ of the Bureau of Plant Industry, U. S. Department of Agriculture.

dried at any other temperature except 98° C. In soy bean leaves stored at −5° C., at which temperature they were not actually frozen, only 81 per cent. of the chlorophyll was retained; but, when the leaves were frozen with Dry Ice, the chlorophyll content of the leaves was unaltered. See table I.

TABLE I
EFFECT OF TEMPERATURE OF DRYING ON CHLOROPHYLL CONTENT OF SOY BEAN LEAVES

SAMPLE NO.	TEMPERA-TURE	CHLOROPHYLL		
		AMOUNT PER GM. OF DRY WEIGHT		AMOUNT RETAINED
		FRESH LEAVES	DRIED LEAVES	
	°C.	mg.	mg.	per cent.
II A ……….	98	22.5	6.8	30
X A ………	72	13.7	12.0	88
IX C ……….	60	21.9	20.8	95
VI C ……….	45	16.5	16.1	98
XII G ……….	30	17.5	16.9	96
II B ……….	20	22.5	15.8	70
II C ……….	5	22.5	17.2	76
IX M ……….	−5	21.9	17.7	81
XII M ……….	−40 to−10	17.5	18.1	103

The results obtained with nasturtium leaves are similar to those obtained with the soy bean leaves and are given in table II. The maximum amount

TABLE II
EFFECT OF THE TEMPERATURE OF DRYING ON CHLOROPHYLL CONTENT OF NASTURTIUM LEAVES

SAMPLE NO.	TEMPERA-TURE	CHLOROPHYLL		
		AMOUNT PER GM. OF DRY WEIGHT		AMOUNT RETAINED
		FRESH LEAVES	DRIED LEAVES	
	°C.	mg.	mg.	per cent.
I A ………	98	21.8	7.1	32
IV A ………	72	23.8	18.8	79
VIII C ………	60	17.3	16.1	93
XIII C .……	45	18.0	15.5	86
VII G ………	30	17.7	15.7	88
I B ……….	20	21.8	17.8	81
I C ………	5	21.8	17.4	80
XIII M …….	−5	18.0	15.7	87
VIII N …,….	−40 to−10	17.3	17.5	101

of chlorophyll retained in the oven dried leaves is 93 per cent., somewhat less than the maximum amount found in the oven dried soy bean leaves. Furthermore, this maximum was found in the nasturtium leaves that were dried at 60° C. rather than 45° C. as was the case with the soy bean leaves. This is in agreement with the optimum temperature for drying plant tissues suggested by MURNEEK (6). The nasturtium leaves dried at room temperature retained about 10 per cent. more chlorophyll than did the soy bean leaves dried at the same temperature. A graphic comparison of the results obtained with the two kinds of leaves is shown in fig. 1.
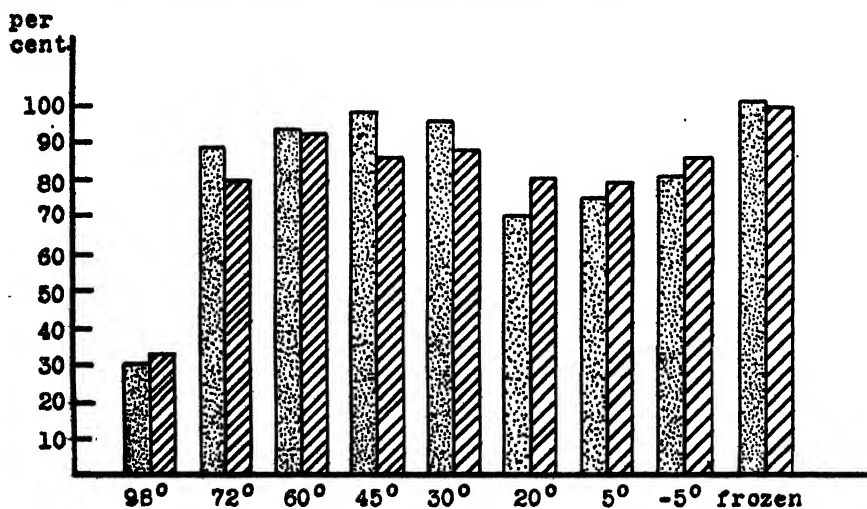
FIG. 1. A comparison of the chlorophyll retained in dried or stored soy bean and nasturtium leaves. The amounts of chlorophyll retained given in per cent. are taken from tables I and II.

■ soy bean.   ☰ nasturtium.

EFFECT OF DESICCATION AT REDUCED PRESSURE

The results of the experiments to show the effect of desiccation at reduced pressure are shown in tables III and IV. Nasturtium leaves dried in an ordinary desiccator over calcium chloride at atmospheric pressure retained only 62 per cent. of the chlorophyll of the fresh leaves, but when desiccated at a pressure approximately equivalent to 27 cm. of mercury, 97 per cent. of the chlorophyll was retained. Leaf samples from the series that was desiccated at the same pressure and subsequently stored in an ordinary desiccator over calcium chloride retained 92 per cent. of their chlorophyll at the end of ten days, and 83 per cent. at the end of 20 days. This seems to indicate that the factors which cause the loss or destruction of the chlorophyll in nasturtium leaves are not entirely eliminated by this treatment. Similar results, however, were not obtained with the soy bean leaves

TABLE III

EFFECT OF VARIOUS METHODS OF DESICCATION ON CHLOROPHYLL CONTENT OF LEAVES

| SAMPLE NO. | METHOD OF DESICCATION | | | SUBSEQUENT STORAGE OVER CaCl$_2$ | CHLOROPHYLL RETAINED | |
	DRYING AGENT	PRESSURE	TIME		SOY BEAN	NASTUR- TIUM
		cm. Hg	days	days	per cent.	per cent.
VII K	CaCl$_2$	76	12	0		62
III G	CaCl$_2$	27	3	0		97
III E	"	27	3	10		92
III F	"	27	3	20		83
V E	CaCl$_2$	27	5	0	89	
V G	"	27	5	10	89	
X E	H$_2$SO$_4$	7	4	0	98	
III H	CaCl$_2$	27	3	Dried at 98° C. 3 day		47

TABLE IV

EFFECT OF DESICCATING LEAVES AT REDUCED PRESSURE IN ABSENCE OF OXYGEN AND CARBON DIOXIDE

| SAMPLE NO. | METHOD OF DESICCATION | | | SUBSEQUENT STORAGE IN ABSENCE OF O$_2$ AND CO$_2$ | CHLOROPHYLL RETAINED | |
	DRYING AGENT	PRESSURE	TIME		SOY BEAN	NASTUR- TIUM
		cm. Hg	days	days	per cent	per cent
VI E	CaCl$_2$	27	4	0	90	
VI F	"	27	4	10	90	
IV 1	"	27	4	0		90
IV J	"	27	4	10		84

desiccated at this pressure. They retained 89 per cent. of their chlorophyll and none of this was lost on subsequent storage. Desiccated at a lower pressure, approximately equivalent to 7 cm. of mercury over concentrated sulphuric acid, soy bean leaves retained 98 per cent. of their chlorophyll. One set of samples, which was dried in an oven at 98° C. after it had been desiccated at reduced pressure, suffered a further loss of about 50 per cent. of its chlorophyll.

The nasturtium leaves that were desiccated at reduced pressure in the absence of oxygen and carbon dioxide retained 90 per cent. of their chloro-

phyll which is somewhat less than was retained by the same kind of leaves desiccated at the same pressure in air. When stored in an atmosphere free from oxygen and carbon dioxide for a period of ten days, they suffered a further loss of chlorophyll.

The results obtained with the soy bean leaves desiccated in the absence of oxygen and carbon dioxide are but slightly different from those obtained with the same kind of leaves desiccated in air at the same pressure. They retained 90 per cent. of their chlorophyll and this was unaltered after being stored for 10 days in the same atmosphere.

FACTORS CONTRIBUTING TO THE LOSS OF CHLOROPHYLL

Although the purpose of the present investigation is to determine the extent to which the chlorophyll is lost in stored leaves rather than to determine the factors which are effective in bringing about this loss, certain of the results obtained are sufficiently suggestive to warrant some speculation as to these causes.

Since only 30 per cent. of the chlorophyll was retained in the leaves dried at 98° C., it seems apparent that this temperature is sufficiently high to bring about the decomposition of the pigment. This is also borne out by the fact that the extracts from these leaves were of a dirty brown appearance rather than the clear green color of pure chlorophyll solutions. Leaves that were desiccated at reduced pressure for three days retained 97 per cent. of their chlorophyll, but when subsequently dried at 98° C., only 47 per cent. was retained. This also indicates that high temperatures are destructive to chlorophyll, but to a less degree if the leaves are practically free from water.

At lower temperatures, however, other factors are probably responsible for the loss of chlorophyll. LINK (4) found that a drying temperature below 65° C. did not check enzymic activity in green succulent tissue. It is stated by LINK and TOTTINGHAM (5) that even in a vacuum oven metabolic changes are inhibited only by a higher temperature (80° C.). The optimum temperature for the action of chlorophyllase as stated by WILLSTÄTTER (10) is about 20° C. Either an increase or a decrease in the temperature will retard the activity of the enzyme and the amount of chlorophyll lost due to this factor will be less. It will be noted that with both the soy bean and nasturtium leaves less chlorophyll was retained in those dried at 20° C. than in the leaves dried at any other temperature (except 98° C.). The amount of chlorophyll retained in the dried leaves increased whether the temperature of drying was increased or decreased. When the leaves were frozen, enzymic activity was probably entirely stopped and the leaves stored in that condition retained all of their chlorophyll. GUTHRIE

(3) also found that frozen leaves retained all of their chlorophyll, but that the ratio between the components (*a* and *b*) was altered. Other factors, such as light and the presence of oxygen and carbon dioxide, may have some effect on the chlorophyll content of stored leaves, but the chief factors contributing to the loss of chlorophyll seem to be temperature and enzymic activity.

Summary and conclusions

Soy bean and nasturtium leaves were dried at various temperatures and desiccated at reduced pressure; they were stored at low temperature in a refrigerator and frozen with Dry Ice. The chlorophyll content of the leaves was determined in the fresh condition and after drying or storage. The amount of chlorophyll retained in the leaves was determined and expressed in terms of per cent. of the chlorophyll content of the fresh leaves. In some cases the results obtained were quite different for the two kinds of leaves used. This would indicate that one might expect still different results if other kinds of leaves were used. For this reason it does not seem wise to draw too general conclusions from the results obtained. The following points are given as a summary of the results obtained with the soy bean and nasturtium leaves.

1. Leaves dried at high temperatures (98° C.) suffer a considerable loss of chlorophyll (approximately 70 per cent.).

2. Leaves dried at room temperatures (18° C. to 24° C.) lost 20 to 30 per cent. of their chlorophyll.

3. The optimum temperature range for oven drying seems to be from 45° C. to 60° C., but even at these temperatures some chlorophyll may be lost.

4. Low temperatures are ineffective in preventing the loss of chlorophyll unless the leaves are actually frozen.

5. Leaves frozen with Dry Ice retain all of their chlorophyll.

6. Leaves desiccated at reduced pressure (7 cm. mercury) over concentrated sulphuric acid at room temperature lost little or no chlorophyll.

7. Leaves desiccated at reduced pressure in the absence of oxygen and carbon dioxide lost more chlorophyll than leaves desiccated in air at the same pressure.

From these results it seems apparent that freezing is the most satisfactory method of storing leaves for later quantitative determination of the chlorophyll. Leaves desiccated at reduced pressure also retain all or nearly all of their chlorophyll, but this procedure is possible only in a laboratory where the extraction and determination of the chlorophyll could also be carried out directly with the fresh leaves.

The writer wishes to express his appreciation to Dr. CARL G. DEUBER who suggested the problem and under whose direction the work was done.

LITERATURE CITED

1. DEUBER, C. G.   Chlorophyll in tree leaves.   Jour. Forestry **27**: 76–77. 1929.

2. GUTHRIE, J. D.   A stable colorimetric standard for chlorophyll determinations.   Amer. Jour. Bot. **15**: 86–87.   1928.

3. —————————.   Effect of environmental conditions on the chloroplast pigments.   Amer. Jour. Bot. **16**: 716–746.   1929.

4. LINK, K. P.   Effects of the method of desiccation on the carbohydrates of plant tissue.   Jour. Amer. Chem. Soc. **47**: 470–476.   1925.

5. —————————, and W. E. TOTTINGHAM.   Effects of the method of desiccation on the carbohydrates of plant tissue.   Jour. Amer. Chem. Soc. **45**: 439–447.   1923.

6. MURNEEK, A. E.   An efficient oven for drying plant material.   Proc. Amer. Soc. Hort. Sci. **25**: 338–341.   1928.

7. SCHERTZ, F. M.   The extraction and separation of chlorophyll (α and β) carotin and xanthophyll in fresh green leaves, preliminary to their quantitative determination.   Plant Physiol. **3**: 211–216. 1928.

8. —————————.   The quantitative determination of chlorophyll.   Plant Physiol. **3**: 323–334.   1928.

9. SPRAGUE, H. B., and SHIVE, J. W.   A study of the relations between chloroplast pigments and dry weights of tops in dent corn.   Plant Physiol. **4**: 165–192.   1929.

10. WILLSTÄTTER, R., and STOLL, A.   Investigations on chlorophyll. (Trans. by SCHERTZ, F. M. and MERZ, A. R.)   385 pp.   The Science Press Printing Co., Lancaster, Pa.   1928.

EFFECT OF FERTILITY ON THE CARBOHYDRATE-NITROGEN RELATION IN THE SOYBEAN[1]

F. A. WELTON AND V. H. MORRIS[2]

(WITH THREE FIGURES)

Introduction

In connection with a study of the cause of lodging in soybeans as grown in corn for silage it was found that the shading by the corn resulted in a reduction of the percentage of carbohydrates and total dry matter, and that the stems of soybeans thus developed were relatively soft, pliable, and weak. In cereals like oats and wheat a similar situation in the stems with consequent lodging has been found to develop as a result not only of shading but also from hyper-nutrition. In the latter case, particularly in the presence of an abundance of nitrates accompanied by suitable moisture relations, there was set up within the oat and wheat plants a low carbohydrate-nitrogen relation characterized by a preponderance of vegetative growth, soft, pliable stems, and consequent lodging. Conversely, under relatively poor soil conditions there was developed a high carbohydrate-nitrogen relation characterized by a preponderance of reproductive growth, hard, rigid, stiff stems, and non-lodging.

Object

The purpose of the work presented in this paper was to ascertain if hyper-nutrition, particularly an abundance of nitrates, affects the carbohydrate-nitrogen relation in soybeans, a legume, the same as it has been shown to affect the carbohydrate-nitrogen relation in other plants such as oats, wheat, and tomatoes.

Material and methods

Soybean plants were grown in square-yard areas on poor, medium, and rich soils. The poor soil was made by mixing one part of Wooster silt loam with three parts of common creek sand which had previously been passed through a sand screen in order to remove stones and any coarse foreign material. The medium soil consisted of Wooster silt loam which was in a good state of fertility. The rich soil was prepared by mixing with one part of the loam three parts of well rotted manure. These three classes of soil were placed side by side and separated from each other by boards one inch thick and seven inches wide. They are henceforth designated in this paper as sand-soil-manure plots.

[1] Contribution from the Department of Agronomy, Ohio Agricultural Experiment Station.

[2] Associates in Agronomy.

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Soybeans were planted in these plots in 1924 and 1925. In 1924 seeds of the Elton variety were planted June 11, putting two seeds in hills spaced one foot apart each way. Samples for analysis were taken September 18 when the plants were well developed, but before the leaves had begun to drop. In 1925 seeds of the Hamilton variety were sown May 29, and samples for analysis were taken September 21.

In sampling, the leaves were removed and the lower two-thirds only of the stems were used. These were cut into pieces one-half to three-fourths of an inch long, placed in wide-mouthed, glass-stoppered bottles, covered with alcohol, the strength of which, after allowing for the moisture contained in the plants, was about 70 per cent., and then heated about an hour at approximately 78° C. The samples were then set aside and allowed to stand for two or three months before analysis.

Aside from total dry matter, analyses were made for certain of the constituents of which the dry matter is composed, namely, free reducing sugars, inverted sugars, easily hydrolyzable carbohydrates, cellulose, and lignin.

Results

The results of the analyses of the plants grown on the three grades of fertility sand-soil-manure in the two seasons, 1924 and 1925, were as shown in the accompanying table.

From the table it may be seen that as the fertility of the soil increased, the dry matter and the total carbohydrates of the soybean stems decreased. Comparing the plants grown in the sand with those grown in the manure, the stems of the former contained more free reducing sugars, more easily hydrolyzable carbohydrates, more cellulose, and more lignin. As far as

TABLE I
EFFECT OF FERTILITY ON COMPOSITION OF SOYBEAN STEMS

MATERIAL	1924			1925		
	SAND	SOIL	MANURE	SAND	SOIL	MANURE
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
Moisture	70.2	72.2	73.2	61.2	63.2	72.0
Dry matter	29.8	27.8	26.8	38.8	36.8	28.0
Total nitrogen	0.7	0.5	0.6	0.4	0.4	0.4
Free reducing sugars	1.0	1.1	0.7	1.4	0.8	0.5
Inverted sugars	0.3	0.3	1.2	0.1	1.5	1.3
Easily hydrolyzable carbohydrates	10.2	10.9	8.8	8.2	9.3	6.2
Cellulose	4.3	3.3	2.9	3.8	3.3	2.5
Lignin	9.8	9.1	7.5	17.6	15.4	10.8
Total carbohydrates	25.6	24.7	21.1	31.1	30.3	21.3

these constituents are concerned, the results are in agreement with those found in certain non-leguminous plants, the tomato (1) and oats and wheat (3) when the latter were grown under similar environmental soil conditions. In the soybean, however, the increase in carbohydrates was not accompanied by a simultaneous decrease in nitrogen as was found to be the case in the non-legumes already mentioned.

The dissimilar response of the soybean may be due to the development of nitrogen-fixing bacteria which live in a symbiotic relationship in the nodules on the roots. LEONARD (2) found that nodule formation in soybeans is closely related to the carbohydrate-forming function of the plant. Since the nitrogen-fixing bacteria are dependent on the host for available carbohydrates as a source of energy, the larger the supply of carbohydrates, the more favorable are the conditions for the fixation of free nitrogen. It may, therefore, be expected that when soybeans are grown under certain conditions, such as in a poor soil, the percentage of nitrogen in the tissues will more nearly approach that found when the plants are grown under conditions which tend to inhibit the accumulation of carbohydrates as in the case of soil high in nitrates. The plants grown in the sand in 1925 showed percentages of nitrogen as high as, and in 1924 higher than, those grown in the manure. Apparently this was made possible through the relatively greater development of roots and nodules in the plants grown in the sand, as shown in table II.

TABLE II

EFFECT OF TYPE OF SOIL, SAND-SOIL-MANURE, ON THE WEIGHT OF SOYBEAN PLANTS, INCLUDING ROOTS, AND NUMBER OF NODULES (7 PLANTS)

KIND OF SOIL	GREEN WEIGHT		PROPORTION OF ROOTS	NUMBER OF NODULES
	TOPS	ROOTS		
	gm.	gm.	per cent.	
Sand	455	30	6.2	379
Soil	508	33	6.1	237
Manure	745	26	3.4	62

The proportion of roots was nearly twice as high in the plants grown in the sand as in those grown in the manure. The growth of tops, however, was relatively small and the stems, like those of the non-legumes to which reference has already been made, were comparatively hard and rigid and not inclined to lodge. The relative size of roots and number of nodules are illustrated in the accompanying figures 1, 2 and 3.

Further evidence to the effect that low fertility does not necessarily result in a low nitrogen content of the soybean was afforded by the analyses
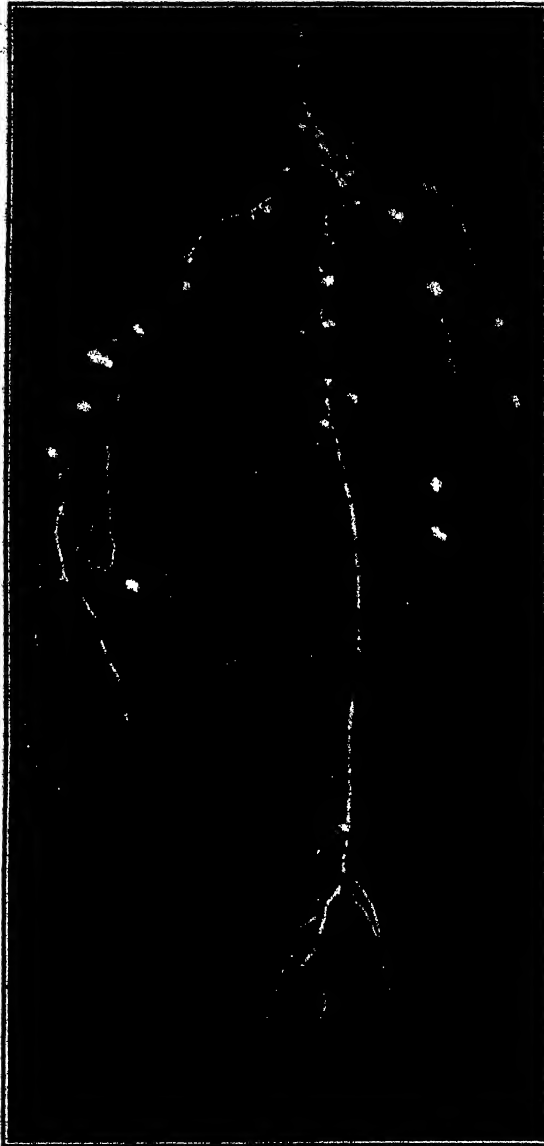
Fig. 1. Root system of soybean grown on sand. Nodules very abundant.

of plants grown in sand and manure in the summer of 1928. Both soluble and insoluble nitrogen determinations were made separately on the leaves and the entire stems. The results obtained were as shown in table III.

Moreover, the kind of soil on which soybeans are grown apparently has little or no effect on the type of nitrogen compounds in the stems and leaves for the percentages of soluble and insoluble nitrogen in the plants grown in
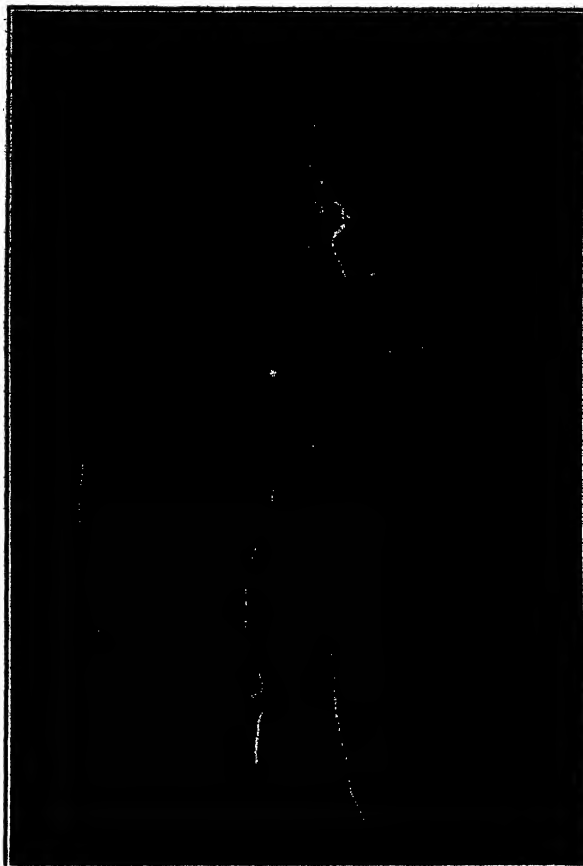
FIG. 2. Root system of soybean grown on soil. Nodules less abundant.

the sand were practically the same as were those in the plants grown in the manure.

Conclusions

Soybeans grown in sand (3 parts of sand to 1 of Wooster silt loam) contained more dry matter and more total carbohydrates than did those

TABLE III
NITROGEN IN SOYBEANS GROWN IN SAND AND MANURE

MATERIAL	SAND			MANURE		
	NITROGEN			NITROGEN		
	SOLUBLE	INSOLUBLE	TOTAL	SOLUBLE	INSOLUBLE	TOTAL
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Leaves	0.13	0.80	0.93	0.09	0.78	0.87
Stems	0.40	0.24	0.64	0.42	0.22	0.64
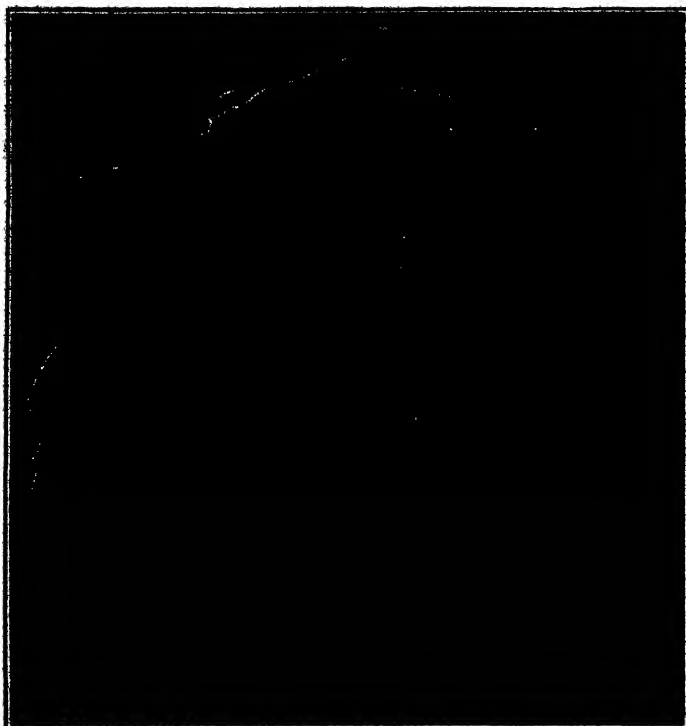
FIG. 3. Root system of soybean grown on manure. Sparse nodule development.

grown in either soil (Wooster silt loam) or manure (3 parts of manure to 1 of Wooster silt loam). In general, the increase was due chiefly to easily hydrolyzable carbohydrates, cellulose, and lignin.

The increase in carbohydrates in the plants grown in the sand was not accompanied by a simultaneous decrease in nitrogen as has been found in the case of certain non-legumes. The high nitrogen content of these plants, however, was associated with the development of relatively large numbers of nodules on the roots of the plants.

The stems of the plants grown in the sand were comparatively tough and rigid and not inclined to lodge.

LITERATURE CITED

1. KRAUS, E. J. and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
2. LEONARD, LEWIS T. A preliminary note on the relation of photosynthetic carbohydrate to nodule formation on soybeans. Jour. Amer. Soc. Agron. 18: 1012–1016. 1926.
3. WELTON, F. A. Lodging in oats and wheat. Bot. Gaz. 85: 121–151. 1928.

WILHELM HOFMEISTER
1824-1877

BRIEF PAPERS

WILHELM HOFMEISTER

FREDRICH WILHELM BENEDICT HOFMEISTER, the son of a proprietor of a music store, publisher, and book-seller, was born at Leipzig, May 24, 1824. The early part of his life was spent as a clerk, with nature study as a hobby. Durng later years he was head of Botany Departments, first at the University of Heidelberg and later at the University of Tübingen. He assumed these positions without university preparation and with only experiences as a self-taught student of botany to recommend him. These experiences, and experiments, began shortly after he left school at fifteen years of age, and led to some of the most profound discoveries ever made in the field of botany. Most of his work was accomplished in the face of the necessity of providing for a large and growing family, and with the physical handicap of poor eyesight, for he was very near-sighted. He pursued the study of plants before and after hours of clerical work and at odd times.

Hofmeister's earlier education was obtained in a private school, the later, in the Leipzig Realschule, where the master taught in a manner that made the pupils think for themselves. He left this school at fifteen years of age to face the world. He early showed an inclination to study nature, largely due to the influence of his father, who was also interested in plants and maintained a small but rather varied, systematically arranged garden. At first he was more strongly inclined towards zoology, but finally decided upon botany. His first salaried position was one in a music store at Hamburg, where in his loneliness he took to roaming the fields. All the while he continued self-education by studying languages, music, physics, chemistry, algebra, geography, etc. Remaining a student to the end, ever trying to improve, he learned Greek in his fortieth year.

After two years at Hamburg he returned to Leipzig and entered into business with his father, where he had leisure time to study. Here he spent sixteen years and did most of the work for which he is famous. At nineteen years of age he had started many fundamental studies that placed him in the front rank of botanists. SCHLEIDEN's Outlines of Scientific Botany awakened his interest in developmental history and microscopic research. He made field trips even outside of Germany.

HOFMEISTER was an excellent teacher for advanced students, rendering excellent service in technique and the preparation of materials, besides being a wonderful incentive to young botanists, and a drawing agency for advanced students. Among these at Heidelberg were: ASKENASY, ENGELMANN, KIENITZ-GERLOFF, N. J. C. MULLER, BOEHM, PFITZER, KRUTITSKY, ROSANOFF, TIMIRIASEV, WOLKOFF, and MILLARDET, and at Tübingen

ZACHARIAS and VON GOEBEL. His quarters were modest. He spent the whole day in the laboratory assisting students. He seemed to be truly indefatigable.

HOFMEISTER was a man of small stature, dark skinned, with vivacious eyes, the quick movements of a southern Frenchman, a very refined character and a kindly humor. He was exceedingly dextrous in the laboratory, near-sightedness assisting in making very good microscopic slides. Poor eyesight was a handicap in other respects, but he was too sensitive about his appearance to wear glasses. This condition frequently got him into humorous positions, as, for instance, greeting every woman he met on the street for fear of appearing to slight some one. His greetings were not mere perfunctory salutes. He was excellent company and possessed a remarkable memory and mind, one that had room for more than the details of his profession. The photograph, plate IX, shows him at the age of 43 years.

HOFMEISTER, in the happy years of a short life, without guiding assistance other than that of his father and occasional visits with botanists, did a monumental work and settled for all time, disputes in regard to certain phases of plant life. His interests and endeavors in plant research were wide and many.

CAMERARIUS in 1695 had shown that sexuality existed in plants but could not explain the fertilization process. KÖLREUTER experimented in crossing plants and demonstrated the presence of inheritance. The details of the process were lacking. In animals LEEUVENHOEK thought the spermatozoa produced the embryo and that the female matured it. SCHLEIDEN thought the tip of the pollen tube became the embryo in a similar manner. He fought stubbornly for this and because of his prestige and gift of argument, really obstructed progress. There were numerous theories of fertilization, of which the above are examples, but when twenty-five years of age, HOFMEISTER settled the question for all time. His technique was so marvelous that in *Oenothera* he could remove the pollen tube from the embryo sac without mutilating either. The publication in 1848 of this work in the *Botanische Zeitung,* entitled "Die Entstehung des Embryo der Phanerogams," is a marvel of direct exposition and is typical of his style. His drawings are numerous, detailed, and exact. This work won him an honorary degree of Ph.D. from the University of Rostock. He afterwards worked on nineteen families of plants. He had seen the egg sac with its egg apparatus and antipodals. His lack of university training was no longer a handicap. The Royal Saxon Society of Science at Leipzig admitted him to membership.

Turning to comparative morphology, HOFMEISTER endeavored to show a continuity through the plant kingdom but seemed to have no thought of the possibility of evolution. He found the function of the spermato-

zoids and archegonia in the liverworts and related it to the other crypto-
gams and phanerogams, and clearly demonstrated the alternation of gen-
erations. JULIUS SACHS gave him full credit for the establishment of the
basis of phylogeny throughout the plant kingdom. HOFMEISTER saw and
recognized the process of fertilization and the liberation of zygospores in
the desmids and diatoms. He noted also many causal things about plant
structure; distinguished between axial, leaf, and hair structures, and
noted their relations. He recognized inheritance, variability, and the mu-
tation of plants.

The controversy in regard to the origin of the cell was waxing hot, be-
fore his call to Heidelberg, with MIRBEL, VON MOHL, SCHLEIDEN, and
NÄGELI furnishing most of the commotion. NÄGELI's concept is the one
commonly accepted today, but HOFMEISTER made many contributions to the
details. He saw nuclear division in an embryo and thought that one-half
of the protoplasm collected around each daughter nucleus. In pollen grains
he saw the nuclear membrane, and saw it and the nucleoli disappear before
cell division, and possibly the cell plate and chromosomes. He recognized
the importance of colloids and their functions in the physical properties of
protoplasm, and studied the permeability of protoplasm. He saw that
protoplasm was most abundant in young cells and that cell growth preceded
cell division, which took place perpendicular to the direction of strongest
growth. Many had considered plants as being composed of independent
units or cells. HOFMEISTER considered the cells as being parts of a corre-
lated structure. He anticipated and saw plasmodesmen, establishing that
plants are made up of united protoplasmic structures. He said cell turgor
was due to osmotic pressure of the cell contents and to water absorption.

HOFMEISTER's experimental physiological work was chiefly on the move-
ment of sap. He noticed that bleeding took place when there was a reduc-
tion of evaporation and that it was not confined to woody plants. He found
a sap pressure of 212 mm. of mercury with the poppy and 461 with
Digitalis. He thought that the tension in the parenchymatous cells and the
guttation of cell cavities forced soil water into the vessels. He discovered
the periodicity of sap movement in vines and the negative pressure in stems.
He observed the curvature of stems due to shock and their subsequent
straightening out. He thought heliotropism was due to a lack of light on
one side which caused greater elongation on that side. The geotropic re-
sponse of roots he thought to be a passive response to gravity, the one big
failure in his observations. He observed that a rise of temperature caused
tulip flowers to open and a decrease caused them to close.

In his twenty-third year HOFMEISTER married the very refined daughter
of a Leipzig manufacturer, who created for him an extremely happy and
beautiful domestic life. His father had built a large house in Reudnitz,

outside of Leipzig, facetiously called by the members of the family and friends, the "Patriarchal Tent," where he and his children's families lived in perfect accord. It was a most happy community in which social relations functioned perfectly and made an ideal setting for Wilhelm's wonderful work. Here he spent sixteen of the happiest and most fruitful years of his life. HOFMEISTER was called to the University of Heidelberg as Professor of Botany in Ordinary, and Director of the Botanical Gardens at the age of thirty-nine. All went well until he moved to Heidelberg, where his wife contracted pulmonary ailments and died a few years later. This was a great blow to him and left him prostrate. Then his youngest daughter also contracted pulmonary ailments and died. This was a further severe shock to him and seriously interfered with his labors. A second daughter died soon afterward. His courage and best powers were gone. At this time dissention appeared in the faculty at Heidelberg. He did his utmost to avoid being drawn into it. On the death of HUGO VON MOHL at TÜBINGEN, he accepted the call to fill his place, but misfortune followed him. Three of his sons had died in infancy, and now the other two, one twenty-one and the other twenty-five, contracted pulmonary troubles, presumably tuberculosis, and were sent to southern France to recuperate. Here they died within a few months. He was unable to attend their funerals since the letter in regard to their condition did not reach him, and the laws of France required burial within twenty-four hours. His grief overwhelmed him. He became morose and dejected, with only an occasional return of his former self. HOFMEISTER was keenly interested in the unity of Germany, and during the Franco-Prussian war seemed to revive, but for only a brief period. With the death of his two sons he saw the passing of his name. He married the daughter of a physician at Lindenau, but there were no children of this marriage. The honor bestowed by the Dutch Society of Science afforded him his last pleasure. He became palsied and lost his speech, an indication of the blows which were to follow. He suffered a stroke of paralysis, from which he recovered enough to resume his lectures for a short time. A second stroke followed, and it was evident that he must retire from his post. Returning to Reudnitz in the fall of 1876, he seemed to revive, but soon suffered another stroke on January 5, from which he failed to recover, dying a week later, on January 12, 1877, in his fifty-third year. Thus closed a brilliant life that under more favorable conditions might have made many more contributions to botanical science.

One must marvel at the immense amount of work of high caliber that HOFMEISTER accomplished in his short life of fifty-three years under adverse conditions. We should especially remember him for explaining the process of fertilization and for laying the foundations of phylogeny.—A. H. LARSON, *University of Minnesota.*

USEFUL DEVICE FOR EVAPORATING ALCOHOL FROM PLANT EXTRACTS

(WITH ONE FIGURE)

Investigators who analyze plant material are always interested in methods which will save them time and labor in carrying out the numerous steps involved in most analytical procedures. In carbohydrate analyses, one of the steps which requires much time and is often troublesome is the removal of the alcohol after extraction with this solvent.

It has been the experience of plant chemists that the removal of alcohol must be complete if subsequent clearing of the solutions is to be satisfactory. Moreover, if certain so-called "biological methods" are to be employed in hydrolysis—as for example, the use of the enzyme emulsin in the determination of glucosides—the extracts must be free of alcohol, since this enzyme is easily inhibited even by low alcohol concentrations. But however thoroughly the alcohol needs to be removed, the process is troublesome and time-consuming. If the plant samples taken for analyses have been preserved in alcohol, instead of being oven-dried, the removal of alcohol becomes even more of a problem.

The method commonly used is to evaporate the alcohol on a steam or hot water bath, either with or without reduced pressure. If evaporation is conducted at atmospheric pressure several hours are generally required and the solutions, at least in the later stages of evaporation, are subjected for a considerable time to a temperature very close to 100° C. Thus, any advantage of using the low boiling solvent, alcohol, for extraction is lost by subjecting the extracted materials to the temperature of boiling water.

If reduced pressure is used the time required to free the extract of alcohol is somewhat shorter, although it is still quite long. The method has an advantage in that the alcohol is recovered and can be re-used. However, at the relatively low cost of commercial alcohol, it is very doubtful if this advantage offsets the numerous disadvantages of the method. The apparatus requires more or less careful attention to insure the proper intake of air to carry off the alcohol vapors and yet prevent splashing of the solutions which would result in a loss of solutes; the flasks occasionally collapse, due to outside pressure and the extract is lost; the rate of removal of alcohol usually does not take place uniformly in the various flasks, necessitating repeated attention from the analyst.

The device reported here is very simple and provides a rapid and satisfactory method of alcohol evaporation. It consists of an air pump driven by a 1/6 H. P. motor, and connected to a piece of 0.75 inch iron water pipe

about 8 feet long into which are fitted, at the proper intervals, 12 curved pieces of 3/16 inch copper tubing each about 7 inches long. The apparatus described accommodates twelve flasks, although for convenience of illustration, a battery of only six is shown in fig. 1.
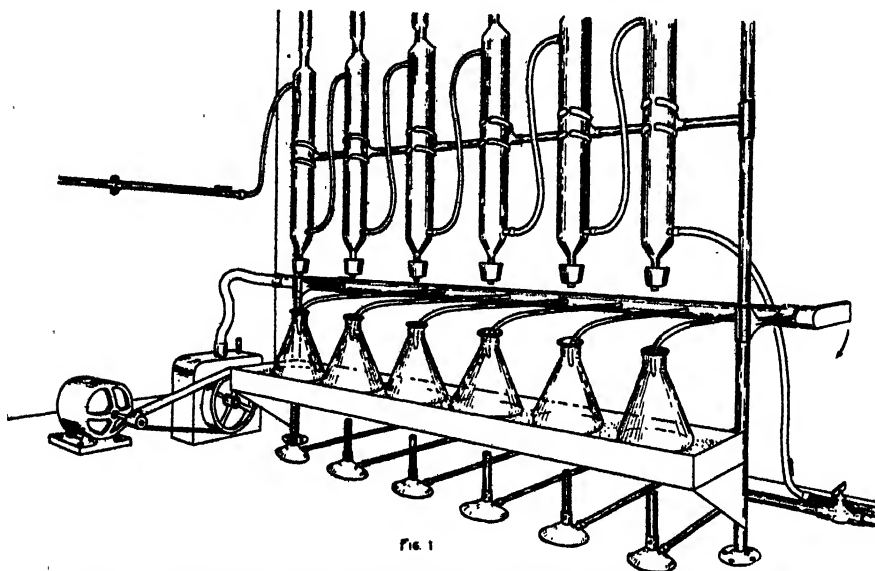
FIG. 1. Device for rapid evaporation of alcohol from plant extracts.

The pump used is a Crowell positive blower, delivering 13 cu. ft. of air per minute. This supply of air, when divided among twelve flasks, is sufficient for rapid evaporation. The pipe carrying the air is fastened in such a way to the back of the stand which holds the condensers that it can be turned in its clamps to swing the copper delivery tubes into the position shown, or back out of the way. A piece of rubber hose connects the pipe with the pump and permits the pipe to turn freely. The apertures of the copper tubes have been reduced by pinching the ends, thus regulating the amount and the force of the air stream from each. A more elaborate arrangement for regulating the air stream could doubtlessly be secured by special fittings for each copper delivery tube.

In order that the Soxhlet tubes can be inserted for extraction, the pipe is turned in its clamps to swing the delivery tubes back out of the way. After extraction all that is necessary is to remove the Soxhlet extractors and turn the pipe so that the delivery tubes enter the mouths of the extraction flasks. The pump is then started and evaporation proceeds at once.

The figure shows a sand bath heated by gas flames. An electric hot plate, of course, serves as well, but if gas is used it should be turned off

when evaporation is started so that the alcohol vapors driven out of the flasks cannot ignite. The residual heat of the sand bath (or hot plate, as the case may be) aids very materially in the evaporation, and yet is not sufficient at the time of complete evaporation to caramelize the carbohydrates in the flasks. This, however, is never a danger as long as the flow of air is maintained. In preliminary work with the apparatus, sugar solutions were evaporated to complete dryness with the gas heat on and yet no caramelization occurred because of the cooling effect of the rapid air stream. In actual work, the extra precaution of turning off the heat is always followed.

Before the pump is started the alcohol in the flasks is at its boiling temperature, but when the flow of air is begun the temperature drops quickly to approximately 35° C. (in the case of 95 per cent. alcohol) due to the cooling effect of rapid evaporation. Near the drying point the temperature may rise to 40° C. The time necessary for the evaporation of 100 cc. of 95 per cent. alcohol is from fifteen to twenty minutes, and even for 80 per cent. alcohol the time is considerably less than one hour. The method is thus seen to effect a rapid evaporation at a low temperature and with a simplicity of operation which requires no special care on the part of the analyst.

Apart from its usefulness in the removal of alcohol, which alone justifies its existence in this laboratory, the device is frequently found useful in various procedures where evaporation is necessary. Its description is presented here with the hope that other investigators can use it to advantage. —F. E. GARDNER, *Department of Horticulture, University of Maryland, College Park, Maryland.*

Seventh Annual Meeting.—The seventh annual meeting of the American Society of Plant Physiologists will be held in Cleveland, Ohio, Dec. 29, 1930–Jan. 3, 1931, in connection with the meeting of the American Association for the Advancement of Science. The headquarters of the Society will be at the Hollenden Hotel, along with the other botanical societies. The call for titles of papers was sent out some time ago, and the Secretary and Program Committee should have the support of everyone in making this a meeting of unusual value.

The annual dinner, Monday, December 29, will be featured again, it is hoped, by the announcement of the second Stephen Hales prize, and the annual award of the CHARLES REID BARNES Life Membership. Members should reserve this evening first, before making any other engagements for dinner programs, or evening lectures.

Cleveland is an interesting, progressive city, and can be depended upon to handle large meetings comfortably. It is centrally located for the northern states. A large and enthusiastic meeting is an inspiration to a greater research program for each individual participant. One cannot afford to miss the dynamic stimulation which comes from meeting one's contemporaries in intimate friendship.

Fifth International Botanical Congress.—The International Congress held at Cambridge, Aug. 16–23, 1930, was a great success. No larger gathering of botanists has ever taken place, and important steps were taken in connection with the work of taxonomists which will have a lasting effect upon botany.

The surroundings of the Congress were ideal, and about 1200 were registered as members, although the number actually in attendance was somewhat less, about 1000 delegates. The meetings were held in the buildings and laboratories of the Colleges of Cambridge University. Examination Hall and the Arts School were used for reception and social purposes during the entire period.

Plant Physiology had probably the largest single section of the Congress, and was credited with about 200 delegates. The next largest group, Ecology, was estimated at 150–200. The meetings of the Physiology Section were held in the central lecture hall on the first floor of the Botany School. The meetings were presided over by Dr. F. F. BLACKMAN, assisted by some of the numerous vice-presidents of the section. There were fourteen of these, as follows: W. L. BALLS, V. H. BLACKMAN, E. DEMOUSSY, H. H. DIXON, P. BOYSEN JENSEN, L. JOST, M. KORCZEWSKI, F. E. LLOYD, H. G. LUNDEGÅRDH, N. MAXIMOW, W. J. V. OSTERHOUT, W. RUHLAND, A.

URSPRUNG, and F. A. F. C. WENT. The recorder of the Section was WAL-TER STILES, and the secretary, G. E. BRIGGS. The vice-presidents were asked to occupy the front row of seats which formed part of a great circle in front of the demonstration table and the President's desk. This was done presumably to lend dignity and impressiveness to the occasion, and to insure that the front seats would be occupied. The vice-presidents discharged this double duty admirably.

The programs were originally outlined for six days, but owing to the absence of all Russian delegates, the papers assigned to Saturday were set back to Friday morning, leaving the final session vacant for more enjoyable purposes, as it was a day of leave-taking. The program on Aug. 18 was devoted to the problems of carbon metabolism, especially respiration, a field to which the Cambridge School, under Dr. BLACKMAN's leadership, has given much attention in recent years. Two of the papers by RUHLAND and BENNETT-CLARK, dealt with the physiology of the organic acids which are related to the carbohydrates.

The following day was given to the consideration of permeability and osmotic behavior of plant membranes. This program was led by JOST's interesting paper on protoplasmic permeability. Prof. PRÁT of Prague showed moving pictures of plasmolysis and swelling of cells, and other studies of permeability were presented by HÖFLER, IRWIN, ÚLEHLA, SHULL, and BRAUNER. The program emphasized the great complexity of permeability phenomena, and the impossibility of explaining it according to any single hypothesis.

The Wednesday program centered about mineral nutrition and growth of higher plants. LUNDEGÅRDH described a spectroscopic method of determining the quantity and kind of ions present in soil or plant solutions, and other papers dealt with phosphoric acid concentrations and growth of corn, mineral nutrition of barley, selective absorption of potassium, and absorption of minerals from insoluble compounds. At 4:30 P. M. Prof. ÚLEHLA presented a splendid moving picture film on plant development at the Cinema Theater. It showed autonomic and tropic movements of seedlings, inflorescences, etc., and was much appreciated.

On Thursday, certain methods of investigating protoplasmic organization were discussed. Bioelectric potentials, hormones, X-ray studies, *intra vitam* staining, and regeneration of woody cuttings were all included under this broad heading. The final sessions took up growth and development of higher plants. V. H. BLACKMAN's high tension electric discharge experiments on barley growth were discussed, and such topics as nutrient ions and enzyme activity, translocation of salts and metabolites, root behavior of cotton, the metabolism of xanthine derivatives, alkaloids, and urea in plants,

and the structure and function of *Utricularia* traps, filled out an interesting and valuable program.

Too much cannot be said in regard to the cordial spirit of the meetings and the social arrangements of the Congress as a whole. In the general plenary meetings, the Congress has, let us hope, found a permanent solution to the species muddle. A compromise was reached, in which the spirit of give and take were approximately equal. All past work in languages other than Latin are validated, but from 1932 on, descriptions are to be in Latin. Concessions were made to American workers in regard to types. As the rules regarding descriptions were passed unanimously, one should have the right to hope that all taxonomists will obey the rules, or leave the description of species to those who will. The settlement of these controversial questions in an amicable manner by the Congress is a great step forward.

The Congress also voted to accept an invitation sponsored by Dr. F. A. F. C. WENT, of Utrecht, to hold the next Congress in Holland in 1935. It is not at all likely that the meetings would be held at Utrecht, but possibly at Amsterdam or Rotterdam, where accommodations would be more adequate.

Membership.—Plant physiologists, or those interested in the literature of plant physiology who desire membership in the American Society of Plant Physiologists, can make application for membership directly to the Secretary-Treasurer, Dr. WRIGHT A. GARDNER, Department of Botany, Alabama Polytechnic Institute, Auburn, Alabama. The membership has become international in character, and plant physiologists in foreign lands are also invited to make use of the privilege of direct application to the Secretary. Members may also send in the names of friends and colleagues who have expressed a desire to become members. The rapid growth of the Society during the last year has now given to PLANT PHYSIOLOGY as large a circulation as the old well established journals. It reaches more plant physiologists than any other botanical journal.

Portraits.—There are now eight portraits in the gallery which is being made available by PLANT PHYSIOLOGY to the members of the Society. They are purchasable at 12 cents each, postage paid. During 1931 it is hoped to continue these portraits, and to add portraits of contemporary plant physiologists chosen from among those who have attained the highest distinction in this field, especially in countries other than the United States.

International Address List.—The second edition of the International Address List was sent out to the members last spring. Some omissions will be noted on looking it over carefully. It does not need to be said that these

omissions are unintentional. Whenever any one finds a name missing, it should be reported, so that future editions can be corrected. To help cover the cost of this bulletin, the Secretary omitted the regular membership bulletin, substituting a mimeographed supplemental list of new members added during the previous year. The International Address List is so valuable that a new edition should be printed every five years, even if we have to forego the annual membership list once in five years.

Errata.—The errors found in the earlier numbers of Vol. 5 of PLANT PHYSIOLOGY are placed at the end of the table of contents in this issue. If the corrections are inserted at the places where the errors occur, it may prevent misunderstanding and misinterpretations later on. Members are urged to correct all of their files in this way. Assistance on the part of authors and readers in detecting errors is invited at all times.

Celebration at Rutgers University.—The Agricultural Experiment Station of New Jersey celebrated its fiftieth anniversary on October 8–9, 1930. Nearly a thousand visitors participated in this happy occasion. Open house was held by the laboratories from 10:00 A. M. to 2:00 P. M., after which there were a number of addresses, and the unveiling of a memorial tablet to the first two directors of the Station, Dr. GEORGE H. COOK, and Dr. E. B. VOORHEES. In the evening a dinner was held in Cooper Hall, with about 475 in attendance. Dr. J. G. LIPMAN, the present Director, was the toastmaster, and Sir JOHN RUSSELL of Rothamsted, and Dr. S. ORLA-JENSEN of Copenhagen were among the after-dinner speakers.

On the second day, a meeting was held in the Elizabeth Rodman Voorhees chapel, at which honorary degrees were conferred upon Drs. C. F. MARBUT, LAFAYETTE B. MENDEL, THEOBALD SMITH, Sir JOHN RUSSELL, S. ORLA-JENSEN, L. O. HOWARD, and CHESTER LATHROP PACK. Following the conferring of the degrees, a luncheon was held for official delegates from other institutions as guests of the Experiment Station staff.

The American Society of Plant Physiologists was represented at the celebration by Dr. JOHN W. SHIVE, who was appointed as the Society's delegate by President H. R. KRAYBILL. The New Jersey Station has an enviable record for aggressive research, and deserves a high place in the history of Experiment Station development in the United States. It has the good wishes of every one of its friends for a greater second half century.

Physical Measurements.—As an aid to the Committee on Physical Methods of the American Society of Plant Physiologists, the Leeds and Northrup Co., 4901 Stenton Ave., Philadelphia, has prepared a special

bulletin. This bulletin was prepared by Mr. MELCHER, and deals with measurements of hydrogen-ion concentrations, electrolytic conductivity, galvanometers, temperature measurements, measurements of radiant energy, humidity measurements, and gas analysis. The bulletin explains the usefulness of various types of instruments, and in case they are not handled by Leeds and Northrup Co., tells where they may be obtained.

They have also reprinted an article by Dr. C. Z. ROSECRANS on "The present state of apparatus for hydrogen ion measurements" from the January, 1930, issue of the Journal of the American Water Works Association. Copies of the special bulletin and of the ROSECRANS reprint may be obtained from Leeds and Northrup Co. on request, by any of our members. The Leeds and Northrup Co. Notebook no. 3 is a valuable text on the subject of hydrogen-ion determinations.

Physiology and Biochemistry of Bacteria.—Volumes 2 and 3 of this work by BUCHANAN and FULMER were mentioned briefly in the July number of PLANT PHYSIOLOGY. Volume 2 is on "The effects of environment upon microorganisms," and volume 3, "The effects of microorganisms upon environment, fermentations and other changes produced."

The authors must have a keen sense of humor to mention in the preface to volume 2 that the treatise is "intended to serve merely as an introduction to the subject." It is hoped that they continue the style and extensiveness of treatment for a complete and exhaustive discussion of Bacteriology, if more is to be given in the present state of knowledge of the subject.

Volume 2 is a thorough presentation of all phases of environmental effects on bacteria, with the physiology and biochemistry fundamental thereto. Students who are able to absorb even a fair portion of the information presented will be well trained in physiology, and biochemistry, as well as in bacteriology. The discussion of the effects of temperature on vital processes in volume 2 is especially good. The discussion of the effects of microorganisms in producing chemical changes in the environment in volume 3 is also excellent. One could not expect books of this kind to be entirely free of errors, but the authors have used an interesting style, the work is carefully compiled, and it is well printed. The bibliographies are quite complete for the subjects covered. The Williams and Wilkins Co., Baltimore, Maryland. Price, $7.50 each volume.

Plant Physiology.—The COULTER, BARNES, COWLES Text Book of Botany is appearing in a new three volume edition, each part paged independently. Volume two, Physiology, is now available, and can be purchased for $1.80 from the American Book Co., 88 Lexington Ave., New York. This volume was revised and enlarged by Prof. C. A. SHULL, of

Chicago. A large number of citations accompany the text, and literature references at the close of each chapter provide suggestions for much collateral reading.

Self Sterility and Hybrid Sterility.—From the press of Julius Springer, Berlin, we have received a copy of volume 21 of the Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere. It is entitled "Selbststerilität und Kreuzungssterilität im Pflanzenreich und Tierreich," and is written by Dr. FRIEDERICH BRIEGER, of the University of Berlin. Following a brief introduction, the work is presented in three sections: A. Parasterility of the higher plants; B. Parasterility of the metazoa; and C. Parasterility of thallophytes and protista. A general discussion of the meaning of self sterility, problems and theories of inbreeding degeneration, and sexuality and parasterility concludes the work. A bibliography of about 700 titles indicates the thoroughness with which BRIEGER has covered the ground. Including the index, the book contains 395 pages. The price of the monograph is 32 RM unbound, and 33.8 RM bound in cloth.

Chemistry of Protoplasm.—The fourth volume of Protoplasma-monographien has been published by Gebrüder Borntraeger of Berlin. It is by Prof. ALEXANDER KIESEL, of the University of Moskau, and is entitled "Chemie des Protoplasmas." There are eight chapters: Protoplasm as a whole; cytoplasm and the chemistry of its morphological structure; cytoplasm and its chemical substances; the nucleus and its constituents; the resting nucleus and division; the chemical substances of the nucleus; non-nucleate cells; and plasmodium of the Myxomycetes as a protoplasmic material. A bibliography of 568 titles is included. A book of this kind impresses the reader in two directions—with how much, and yet how little —we really know about the chemistry of the fundamental living machinery of animals and plants. The price of this monograph is 20 RM, and orders should be addressed to the publishers, Berlin W 35, Schöneberger Ufer 12a.

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